The evolution and diversity of the Anolis dewlap

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Abstract

The neotropical lizard genus *Anolis* is an important model system for studies of the ecology and evolution of animal diversity. One of the most striking elements of *Anolis* diversity is found in the morphology of the dewlap, an extensible flap of colored skin on the throat that anoles use to communicate during social interactions. The evolutionary forces that have promoted the evolution of dewlap diversity are poorly understood. A study of reproductive success in *A. carolinensis* showed for the first time that dewlap color is currently under selection in an anole (Chapter 1). However, this is unlikely to be a result of intrasexual competition because neither dewlap morphology nor reproductive success are related to male territory size or quality. Instead the dewlap may be under intersexual selection from female mate choice. In addition to sexual selection, the dewlap may evolve in response to a variety of other processes such as species recognition, predation, sensory drive, or a combination of these. A study of variation among populations of a single species, *A. sagrei*, revealed that the dewlap may be undergoing rapid adaptive diversification driven by several of these processes simultaneously (Chapter 2), while a study of variation among species in dewlap size showed that similar processes are likely shaping the evolution of the dewlap in female anoles (Chapter 3). In a case study of male-female pair formation in the Costa Rican anole *A. limifrons*, dewlap size or color were not good predictors of which males would form pairs and which would not, though males and females that were similar in size were found to form pairs more often than animals that were dissimilar in size (Chapter 4). Finally, a study of the correlated evolution of traits related to locomotion in anoles found that morphology, behavior, and habitat use evolve in tandem among 31 species of anoles from the Greater Antilles (Chapter 5). Together, these studies suggest that the evolutionary ecology of

anoles is more complex than previously thought, and that future studies of the dewlap may provide more general insight into the evolution of diversity of animal ornaments.

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Chapter 1

Introduction

The neotropical lizard genus *Anolis* has played a critical role in the advancement of evolutionary ecology, almost since the creation of the field. While most people are likely familiar with just two species, *A. carolinensis* and *A. sagrei*, because they are widespread in pet stores, at carnivals, or on Florida palm trees, anoles are one of the most diverse vertebrate genera. At last count, 387 species of anoles were listed on the Reptile database, a website devoted to cataloging reptile diversity worldwide, and 39 of these have been described since 2000 (Uetz, 2014). In fact, according to the database, the rate of new species described per decade has held steady at about 30 since the 1960s and shows no signs of slowing.

The diversity of anoles is not just reflected in the large number of described species, but in their variety of shapes and sizes (Losos, 2009). The largest anoles are as long as a human forearm, while the smallest look diminutive even on a pinky finger. Some anoles are bright green giants, while others are tiny and blotched white like a branch covered in lichen. Some have long spindly legs or tails twice as long as their bodies, while others have short tails that can grasp branches and stubby legs held close to their bodies. Some have fins running along their tails like primeval dinosaurs, and others have crests on their necks that inflate like balloons when they fight. Of all the morphological variety you can find in anoles, the most impressive variety is found in the dewlap, a loose flap of skin that can be stretched out over the cartilage of the throat like a flag during displays. The dewlap comes in virtually every color humans can imagine (Figure 1.1) and many that we can't since anoles, like birds, can see ultraviolet light (Fleishman, 1992).

Figure 1.1. A sample of variation among species of *Anolis* in the size, color, and pattern of the male dewlap. Species from left to right, top to bottom are: *A. tolimensis*, *A. unilobatus*, *A. ventrimaculatus*, *A. auratus*, *A. bombiceps*, *A. gadovi*, *A. sulcifrons*, *A. isthmicus*, *A. aquaticus*, *A. rupinae*, *A. tropidogaster*, *A. uniformis*, *A. microtus*, *A. aequatorialis*, *A. latifrons*, and *A. lyra*. Photos courtesy of Luke Mahler and Steven Poe.

Anoles have been the focus of a extensive research on a broad array of topics in evolutionary ecology. According to Google Scholar, over 5,000 studies have been published with *Anolis* in the title as of August 2014, and of these over 1,500 have been published in the last five years. The topics of these studies have spanned a breadth of topics, such as behavior, phylogenetics, functional morphology, reproductive physiology, interspecific competition, convergent evolution, intraguild predation, biogeography, community structure, and speciation, to name a few. Many of these studies have considered the Anolis dewlap specifically: of the *Anolis* studies

noted above, 631 contained the word "dewlap" in the title, and 191 of these were published in the last five years. Even though substantial effort has been devoted to the study of the dewlap, there is no consensus on why the dewlap is so variable among species. In fact, it is not clear what forces shape dewlap evolution even within one species, let alone the diversity between them.

On the one hand, the dewlap has all the hallmarks of a sexually selected trait: it is conspicuous, more developed in males than in females, and displayed during male territorial contests and courtship of females. In fact, the *Anolis* dewlap was even discussed as an example of a sexually selected trait on Darwin's original work outlining the theory of sexual selection (1871). Describing a contest between two male *A. cristatellus*, Darwin described:

"*On first seeing one another, they nod their heads up and down three or four times, at the same time expanding the frill or pouch beneath the throat* [the dewlap]*; their eyes glisten with rage, and after waving their tails from side to side for a few seconds, as if to gather energy, they dart at each other furiously, rolling over and over, and holding firmly with their teeth*."

On the other hand, there is mixed support for sexual selection on dewlaps. Some studies have supported Darwin's conclusion that sexual selection is responsible for dewlap evolution. There is evidence for both intersexual selection, typically in the form of female mate choice, and intrasexual selection, typically in the form of male competition for access to mates via control of exclusive territories, in *Anolis*. Intersexual selection is suggested by the display of the dewlap during courtship, and the observations that sexually receptive *A. carolinensis* females prefer to associate with males with functional dewlaps (Greenberg and Noble, 1944), and that they prefer pink dewlaps (the natural color in this species) to green (Sigmund, 1983). Intrasexual selection is suggested by the display of the dewlap during male-male territorial contests (Gorman, 1968; Rand and Williams, 1970; Trivers 1976), and dewlap size appears to be an indicator of bite force (Irschick et al., 2006; but see Henningsen and Irschick, 2011; Lailvaux et al., 2012).

Other studies have contradicted the idea that sexual selection is an important force in driving dewlap evolution. Males with their dewlaps disabled or reduced in size are no less likely than intact males to win territorial disputes, both in the lab and in the field (*A. sagrei*: Tokarz, 2002; Tokarz et al., 2003; *A. carolinensis*: Henningsen and Irschick, 2011). And males with disabled dewlaps are able to attract mates: in field and lab studies they were found to copulate as frequently as intact males (*A. sagrei*: Tokarz, 2002; Tokarz et al., 2003; Tokarz et al., 2005). Studies have also failed to demonstrate female preference for or against a naturally occurring white-dewlapped variant of *A. carolinensis* found in a restricted area in Southwestern Florida (MacDonald and Echternacht, 1991), and gene flow between populations with different dewlap colors in two other species is high, suggesting the absence of assortative mating driven by female preferences (Ng and Glor, 2011; Stapley et al., 2011).

In chapter 2, I test the predictions of the sexual selection hypothesis by measuring current selection on dewlap morphology in a population of *A. carolinensis,* and determining if dewlap morphology (and male reproductive success) is correlated with male success in intrasexual territory competition. I find evidence that selection is currently acting on dewlap color, but not by mediating the outcome of male competition. These results suggest that dewlap color may play a role in female mate choice.

Aside from sexual selection, several other mechanisms of selection have been proposed to act on the dewlap. Perhaps the most commonly discussed hypothesis is that the dewlap facilitates species recognition (Rand and Williams, 1970; Losos, 2009), which some authors consider an extension of mate choice (Ryan and Rand, 1993; Mendelson and Shaw, 2012). The most direct support for the role of the dewlap in species recognition comes from experimental data showing that male *A. cybotes* are more agressive to the closely related sympatric *A. marcanoi* when their

dewlap is painted to resemble that of *A. cybotes*, and vice versa (Losos, 1985). Another compelling pattern suggesting that the dewlap facilitates species recognition comes from *A. caudalis*: where this species overlaps with the orange-dewlapped *A. websteri* in the north of its range, it has a white dewlap with a small orange central spot; where it overlaps with the white-dewlapped *A. brevirostis* in the South, it has an orange dewlap with a thin white edge; and in between it shows a gradient between these extremes (Webster and Burns, 1973). More indirectly, a species recognition role for the dewlap is suggested by the greater information content of the dewlap in complex *Anolis* communities, and the observation that sympatric species almost always differ in several aspects of the appearance of the dewlap (Rand and Williams, 1970; Nicholson et al., 2007).

Sexual selection and species recognition could operate simultaneously on the same trait, and their interaction could be important in driving ornament diversification. For example, dewlap diversity that originates as a consequence of differences among populations in the strength of sexual selection might subsequently lead to reproductive isolation if the same elements of the dewlap function as species recognition cues.

Predation could also generate selection on the dewlap, either independently or in concert with sexual selection and species recognition. When anoles display their dewlap to conspecifics or congeners, they risk the signal being detected by predators, and more conspicuous signals are more likely to be detected (Steinberg et al., 2014). When predation pressure is strong, the risk associated with displaying could lead to selection for smaller, less conspicuous ornaments or less frequent, less flamboyant displays (and in fact anoles reduce the motion of their displays in the presence of predators: Cantwell and Forrest, 2013; Driessens et al., 2013; Steinberg et al., 2014). If predation is interacting with selection for species recognition or sexual selection, the dewlap

may face conflicting demands. In this scenario, changes in the overall intensity of predation, or in the identity of predators could shift the cost-benefit ratio of colorful displays, leading to changes in dewlap morphology among populations.

An alternative idea related to predation is that anoles may use their dewlap to signal to predators that they are aware of their presence and are unlikely to be easily captured, thus deterring the predators from attempting an attack (Caro, 1995). The pursuit deterrence hypothesis is supported by behavioral observations in anoles, which often display in response to the simulated approach of a predator (Leal and Rodruiguez-Robles, 1995; 1997; Leal, 1999). Unlike the classical view of a conflict between predation and sexual selection, pursuit deterrence could potentially favor larger or more conspicuous dewlaps if they are more effective at communicating to potential predators that the anole is aware of the predator's presence, or if such ornaments indicate that the anole in unlikely to be an easy target (for example, if the dewlap indicates running ability or bite force). Contrary to the classical view, pursuit deterrence could lead to larger or more colorful ornaments at sites with high predation pressure if the benefits of pursuit deterrence signaling outweigh the greater risk of drawing attention when these ornaments are used in other contexts.

A final idea that has been proposed to explain dewlap color variability is sensory drive: the specific demands of transmitting a visual signal depend on the visual characteristics of the environment, so species that inhabit different environments will evolve differences in dewlap color or size to maximize signal transmission in their particular habitat, regardless of the identity of the intended signal recipient (Endler, 1990; 1992). Anoles inhabit a wide variety of habitats that differ in the spectrum of light filtering through the vegetation and the average background color against which the dewlap will be viewed, so the dewlap color that will most effectively

attract attention in a given environment is likely to vary among habitats (Losos, 2009). For example, in closed canopy forest where the little light that penetrates the trees has first been filtered through leaves, pale colors will appear more conspicuous than dark ones (Endler, 1993). If populations occupy different habitats, their dewlaps may diverge enough to lead to the formation of new reproductively isolated species (though reproductive isolation will depend on the dewlap also as a species recognition cue). As predicted, species that occupy open habitats differ in dewlap color from species that occupy closed habitats (Fleishman, 1992; 2000), and within species, population-level variation in dewlap color mimics this pattern (Leal and Fleishman, 2004; Ng et al., 2013).

Sexual selection, species recognition, predation, and sensory drive are not mutually exclusive hypotheses to explain dewlap variability, and all of these processes may be acting simultaneously across the genus *Anolis*, both to shape the dewlap within species and to promote variation among species. The relative importance of each of these processes and how they interact with each other is currently unknown (Losos, 2009).

The focus of chapters 3 is to assess the correlation between ecological variables that may reflect each of these processes and male dewlap variation among recently introduced populations of *A. sagrei*. I find that different aspects of dewlap variation among populations are strongly predicted by each of these variables. More surprisingly, I find that these recently established populations are highly variable, most likely as a result of rapid adaptive evolution to the novel environments where they occur. In chapter 4, I use a similar approach to look at the evidence for these hypotheses in a comparison among 227 species of *Anolis*, in this case focusing on one element of dewlap phenotype, dewlap size, and focusing on female anoles instead of males. In

this case, I find some support for sensory drive and sexual selection, but not for species recognition.

In chapter 5, I depart somewhat from a focus on the evolution of the dewlap to consider an unusual case study, *A. limifrons*. Unlike the majority of anoles, which have promiscuous or polygynous mating systems, *A. limifrons* form lasting pairs in nature (Talbot, 1985). I collected data on the morphology of both males and females that were found in pairs and solitary individuals to determine what aspects of morphology are important in pair formation. Animals found in pairs were no different from solitary animals in body size, limb dimensions, head dimensions, or dewlap size. Within pairs, animals mated assortatively by size, such that large males tended to pair with large females and small males with small females. This pattern could be a consequence of a number of different processes, discussed at length in the chapter.

Chapter 6 departs from consideration of the *Anolis* dewlap entirely to consider habitat, morphological, and behavioral diversity among species in traits related to arboreal locomotion. In this chapter, I tested the predictions of the habitat matrix model, a model relating the demands of different modes of locomotion to variation in morphology and habitat (Moermond, 1979a; 1979b; Pounds, 1988; Losos, 1990). I use a phylogenetic canonical correlation analysis to test for correlated evolution between three classes of variables: locomotor behavior, microhabitat use, and appendicular morphology. I find broad support for the model, including the prediction that species using narrow branches will have short limbs and will seldom run, and that species on low perches have long hindlimbs and tails and jump between perches frequently.

Taken together, these studies suggest that our previous understanding of social behavior in anoles may be incorrect, and further research is needed to determine how anoles maximize their reproductive success in a natural setting. The morphology of anoles, in particular the

morphology of the dewlap, is likely subject to many competing types of selection, and tradeoffs between these forces may have contributed to the impressive diversity of this genus. Future studies should focus on measuring different types of selection in detail, preferably in wild populations under natural conditions. Although anoles have been the subject of much research in the past, my results suggest that we still have much to learn from the study of these remarkable lizards.

Chapter 2

Individual variation in dewlap color and reproductive success in male *Anolis carolinensis*

Abstract

Sexual selection is often assumed to drive the evolution of sexually dimorphic animal ornaments; nonetheless, the empirical record of studies measuring selection on ornaments frequently shows ambiguous results. The *Anolis* dewlap is one example of an ornament that is putatively sexually selected despite mixed evidence from lab and field studies. We tested the hypothesis that the size and color of the dewlap of male *Anolis carolinensis* are currently under selection by quantifying the relationships between dewlap morphology, territory, and reproductive success of 58 resident *A. carolinensis* males in a wild population in northeast Florida over four months during the summer of 2008. We found that dewlap color is under selection: males with less red and more UV, green, and blue reflectance in their dewlaps had more offspring than other males. Dewlap traits did not predict male home range characteristics nor did male home ranges predict reproductive success, suggesting that the dewlap is under selection for some purpose other than intrasexual territorial contests between males. The idea that territorial contests are critical to male reproductive success was also contradicted by our observation that members of a parent pair were often located at large distances from one another and that many females mated with multiple males. We suggest that intersexual selection is likely occurring in this species and should be a focus of future studies of the *Anolis* dewlap. The color of the dewlap is correlated with male size and condition, as well as the presence of parasites, suggesting that females could use dewlap color as an indicator of male quality when making

reproductive decisions. Finally, we observed a large number of non-resident, transient males in our population; these males may represent a previously unappreciated strategy or phenomenon in anoles, deserving further study.

Introduction

Animal ornaments, morphological structures used for visual signaling, come in a dizzying variety of forms and colors, from the impressive fins of swordfish to the colorful plumage of the birds of paradise and the elaborate pigmentation patterns on the wings of Hawaiian *Drosophila* (Diamond, 1981; Kaneshiro and Boake, 1987; Basolo, 1990). Sexually dimorphic ornaments used during courtship or male competition are often assumed to function in sexual selection (Darwin, 1871; Andersson, 1994), yet in many cases the evidence linking ornament morphology to fitness is lacking, either because this assumption is never tested or because tests of selection on ornaments yield ambiguous results (Johnstone, 1995; Olsson and Madsen, 1995; Kingsolver et al., 2001; Cornwallis and Uller, 2010; Sousa and Westneat, 2013).

One ornament with unclear fitness consequences is the *Anolis* dewlap. This extensible flap of colorful skin on the throat has been called "a reptilian equivalent of the plumage and song of birds" (West-Eberhard, 1983) because it is typically conspicuous, sexually dimorphic, and is displayed frequently during social interactions. Various studies in the lab and the field, reviewed below, have suggested that the dewlap is a sexually selected ornament. However, despite decades of effort, researchers have found no clear link between dewlap morphology (size, color, or pattern) and reproductive success.

The dewlap, if it is sexually selected, may function in either intra- or intersexual selection. Support for intrasexual selection comes from observations that the dewlap is extended during

male territorial contests (Darwin, 1871; Gorman, 1968; Rand and Williams, 1970; Trivers 1976), and that the dewlap appears to honestly indicate male bite force in *A. carolinensis* (Irschick et al., 2006; but see Henningsen and Irschick, 2011; Lailvaux et al., 2012). However, experimental evidence contradicts this conclusion: when the dewlap is experimentally disabled or reduced in size for both *A. carolinensis* and *A. sagrei*, males are equally able to defend territories or win staged contests against other males (Tokarz, 2002; Tokarz et al., 2003; Henningsen and Irschick, 2011).

Likewise, there is mixed evidence for intersexual selection on the dewlap. Males extend their dewlap during courtship, sexually receptive females prefer to associate with males with functional dewlaps (Greenberg and Noble, 1944), and female *A. carolinensis* show a preference for males with natural pink dewlaps over green-painted dewlaps (but only at a distance: Sigmund, 1983), suggesting that the dewlap may function in male-female interactions. The dewlap also appears to stimulate females to reach sexual maturity more quickly (Crews, 1975), although in this case changing the color of the dewlap did not affect female development. Yet, female *A. carolinensis* from a naturally occurring population with grey dewlaps do not show a consistent preference for males with grey or pink dewlaps, nor do females from "normal" pink populations show a preference for or against grey-dewlapped males (MacDonald and Echternacht, 1991). Additionally, adjacent populations of *A. distichus* in similar habitats that differ in dewlap color do not show evidence of reduced gene flow, as might be expected if females had a preference for males from their population of origin (although adjacent, distinctively colored populations in *dissimilar* habitats did show reduced gene flow: Ng and Glor, 2011). Likewise, two dewlap color morphs of *A. apletophallus* do not show reduced gene flow where they co-occur (Stapley et al., 2011). The importance of the dewlap in intersexual

selection is also contradicted in another species, *A. sagrei*: males with experimentally disabled dewlaps copulated as often as males with intact dewlaps, both in the lab and in the field (Tokarz, 2002; Tokarz et al., 2003; Tokarz et al., 2005).

Another argument against intersexual selection comes from studies suggesting that female lizards appear to choose territories rather than males and mate almost exclusively with the male that controls their territory, a mating system referred to as resource-defense polygyny (Emlen and Oring, 1977; Tokarz, 1995; Olsson and Madsen, 1995). The implication is that even if female anoles show a strong dewlap preference in the lab, this preference may not translate into differential fitness of males in the wild because female anoles do not choose among competing males (*A. carolinensis*: Ruby, 1984; Nunez et al., 1997; Jenssen and Nunez, 1998). Despite some evidence suggesting that females do not mate exclusively with male territory holders (*A. garmani*: Trivers, 1976; *A. valencienni*: Hicks and Trivers, 1983; *A. carolinensis:* Passek, 2002), the majority view among anole biologists is that male territories are the main determinant of male access to females (Orrell and Jenssen, 2003; Losos, 2009).

Taken together, these contradictory lines of evidence for sexual selection led us to investigate two hypotheses about the relationship between dewlap size, color, and fitness in *A. carolinensis*. First, we hypothesized that the size or color of the male dewlap is currently under selection, which has never been demonstrated because previous studies have not directly measured reproductive success. If this hypothesis is correct, male reproductive success will be correlated with dewlap size or color. Second, we proposed that dewlap size and color influence male reproductive success by mediating the outcome of intrasexual contests for territory between males. If the dewlap is functioning in intrasexual selection, we predicted a correlation between dewlap morphology and territory (size or quality), and a correlation between territory and

reproductive success. Alternatively, if the dewlap is under selection for some function other than mediating territorial disputes, such as intersexual selection, we predicted that dewlap morphology will not correlate with territory size or quality, but will correlate with reproductive success.

To investigate these hypotheses, we measured the dewlap size and color, home range (as an approximation of territory), and reproductive success for adult *A. carolinensis* in a single population in northeastern Florida over four months during the spring and summer of 2008. The large number of individuals measured and genotyped in this study make our dataset well-suited to detecting evidence for selection on the male *Anolis* dewlap, if such selection is currently acting.

Materials and Methods

To determine how dewlap morphology interacts with fitness for male *A. carolinensis*, we examined the relationships between morphology and dewlap color, dewlap morphology and home range variables, and all variables and reproductive success in a population in Northeastern Florida. We marked and measured nearly all adults in this population before conducting focal observations to estimate the home range of individual males. We estimated male reproductive success by collecting tissue samples from adult males, females, and juveniles in the population and genotyping them to determine parentage for a large fraction of the juveniles.

Field data

We sampled adult and juvenile green anoles, *Anolis carolinensis,* at the Guana-Tolomato-Matanzas National Estuarine Research Reserve in Florida from May-August 2008. Sampling occurred in 0.69 hectares of riparian habitat dominated by bladderpod (*Sesbania vesicaria*) at the

edge of a pond surrounded by live-oak hammock (Figure 2.1a). An animal was considered an adult if its snout-vent length exceeded 43 or 45 mm (for males and females, respectively) while smaller animals were considered sub-adults or juveniles (Gordon, 1956).

Individual anoles were captured by hand or noose, both during daylight hours and at night while the animals were sleeping. For each adult, we recorded the capture site to 3 m accuracy by GPS (Garmin eTrex Vista HCx) and marked the site with biodegradable orange flagging to ensure the animal was returned to the exact site of capture. Each adult was given a unique identification number and permanently marked with a subcutaneous VIE alpha tag (Northwest Marine Technologies) under the skin of the right thigh and temporarily marked with a queen bee tag glued to the dorsum over the pelvis (Figure 2.1b; Johnson, 2005). We did not individually mark juveniles because our tags were too large, but we released them in suitable habitat about a mile from the study site to prevent re-sampling (genetic markers were later used to confirm that individual hatchlings were not resampled).

We estimated the proportion of adults in the population by sampling over three consecutive days at the end of the field season. On those days, all adults sighted were captured and recorded as either marked or unmarked (Schoener and Schoener, 1982). The average number of unmarked animals over three days allowed us to estimate that we had sampled 95.4% of the adult population that we had sampled. We believe this estimate is conservative because unsampled animals collected on these days could represent natural levels of juvenile recruitment, animals that were not present as adults at the start of the study because they had not reached reproductive maturity, or migration.

Figure 2.1. (a) Photograph of the field site: a shallow pond surrounded by tropical live oak hammock at the Guana-Tolomato-Matanzas National Estuarine Research Reserve in St. John's county, Florida. The zone between the live oak and the pond was dominated by bladderpod (*Sesbania vesicaria*). The apparent abundance of insect life surrounding the pond may have led to relatively high densities of *A. carolinensis*, while the bladder pod made capturing anoles easy. **(b)** An adult male *A. carolinensis* perched on bladderpod, marked with a queen bee tag for minimally invasive behavioral observations.

After capture, we transported animals to the lab in cloth bags for 24 hours. For all captured animals, we recorded snout-vent length (SVL, a standard measure of size for lizards) and mass for each animal using digital calipers and a 15 g Pesola scale. We used mass to estimate the body condition of each individual using standardized residuals from a regression of mass on SVL (Jakob et al., 1996; Schulte-Hostedde, 2005; but see Green, 2001 for potential pitfalls of this approach). Additionally, we recorded the presence or absence of ectoparasites as a binary trait, as parasites have been correlated with coloration in a variety of species (Hamilton and Zuk, 1982; Read, 1987; Lozano, 1994). All measurements were made by A. Harrison to control for individual differences in measurement technique.

Each adult male was photographed with the dewlap fully extended from the left side against a standard 17% grey background (WhiBal®, PictureFlow LLC) with scale bar using a Canon

Rebel Xti digital SLR camera. The reflectance of the dewlap was also measured at three sites on the dewlap—in the anterior region, the posterior region, and near the ventral edge—using an Ocean Optics USB2000 spectrophotometer with a pulsed-xenon light source.

We sampled tissue from both adults and juveniles by pulling ~10 mm from the tip of the tail (enabling the tail to break along natural fracture planes) and stored all tissues in 95% ethanol. After measuring, photographing, and collecting tissue, animals were released within 24 hours of initial capture.

Behavioral observations

Home range size and quality have long been considered to be important to the fitness of individual anoles (Ruby, 1984; Jenssen et al., 1995; Jenssen and Nunez, 1998). In *A. carolinensis*, the size of the home range is approximately the size of the territory (the portion of the home range that is defended [Gorden, 1956]); thus, we used our home range estimates as approximations of the territory size for individual males. To estimate home range sizes, we established a reference grid throughout the study site with flags placed at 5 m intervals radially around the edge of the pond. We measured the GPS coordinates of these flags and used them subsequently to geo-reference the recorded locations of lizards during focal observations.

Home ranges were estimated for adults using 30-minute focal observations conducted between 10:00 am-1:00 pm and 3:00-6:00 pm, when the lizards were most active. After we identified an animal for observation (by reading the queen bee tag with binoculars), an observer sat 3-6 m away from the individual and allowed the animal to acclimate to the observer's presence for five minutes. Following the acclimation period, we recorded the animal's location throughout the 30-minute trial in a hand-drawn map of the home range that included at least three points from the reference grid. We also recorded the position of additional animals on the

same map if they were easily identified and in the same general area as the focal animal. We did not conduct more than one observation session per animal per day. To optimize our effort, we conducted observations on each section of the study site by moving counter-clockwise around the pond each day.

Focal observation data was translated into home range size estimates by scanning the hand-drawn maps and geo-referencing each using the reference grid points on the map. We then extracted the location of each observation from the map, and used these points to calculate the minimum convex polygon constituting the individual's home range and its size using the Hawth's tools v3.27 with ArcMap v9.3 (Beyer et al., 2004; ESRI, 2008). We also calculated the number of female and male neighbors within 5 m of a focal animal's home range centroid, based on Gordon's (1956) territory diameter estimate 6-7m for two populations of *A. carolinensis* in Louisiana, and a 4m estimate of the gap between male territories in a Georgia population (Jenssen et al., 1995). A principal components analysis was used to define two principal component axes that captured variation in territory quality as the number and ratio of male and female neighbors.

Additionally, another important prediction of the hypothesis that male territories are key to male reproductive success is that males will mate with females within their territories. To test this, we calculated the distance between male-female pairs that successfully reproduced by measuring the linear distance between territory centroids (or initial GPS coordinates if no home range estimate was available) for all parent pairs that were identified in our parentage analysis (described below). This distribution was compared with the distribution of distances between all males and females within the population using a Student's t-test.

Our observational effort was not equal for all individuals, meaning that we had more observations for some individuals than others. The number of observation points was positively correlated with the estimated size of the home range ($r = 0.56$, $p < 0.001$). We corrected for this effect by ln-transforming home range area to make it a linear variable, and then regressed transformed area on number of observations. We used the residuals of this regression as our relative home range estimates in the final analysis (using uncorrected territory estimates did not qualitatively change any of the results).

Dewlap characteristics

Male dewlap size was estimated from photographs by measuring the area of the extended dewlap using ImageJ v1.44 (Abramoff et al., 2004). We measured each dewlap five times per photograph and took the average of these measurements to calculate total dewlap area. These measurements were scaled to the length of a 1cm reference bar included in each photograph. To separate body size from dewlap size, we calculated relative dewlap size by regressing ln-transformed dewlap size on ln(SVL) and using residual dewlap size in our analyses. Dewlap color was characterized from spectral reflectance measurements. First, spectral data were smoothed and condensed by averaging reflectance at 5 nm intervals across a length of the spectrum that captures the expected range of visual sensitivity for anoles (300-700 nm) using the program CLR v1.05 (Montgomerie, 2008). We then calculated the average reflectance profile for each individual from the three measurements taken.

The reflectance curves were analyzed using the specifications of the *Anolis* visual system to find color variables that capture variation that anoles are able to detect. To accomplish this, we calculated the relative stimulation of each of the four photoreceptor types (LWS, MWS, SWS, and UVWS) found in the *Anolis* retina. We did this by multiplying the reflectance spectrum of

the dewlap by the sensitivity curve of each photoreceptor type similar to the methods used by Fleishman and Persons (2001; see also Fleishman et al., 2009). The photoreceptor sensitivity curve was estimated by using peak sensitivity values for *A. carolinensis* from Loew et al. (2002) fit to Lamb's photopigment template (Mansfield, 1985; Lamb, 1995). The sensitivity curve was then transformed to reflect the absorption spectrum of the oil droplet most commonly found in association with the photoreceptor type by setting sensitivity to zero below the mean 50% cutoff value for that photoreceptor using values from Loew et al. (2002). We transformed the sensitivity functions so that all receptor types were weighted equally, that is, so the area under each curve summed to one. This method produced five species-relevant dewlap color variables: brightness (all photoreceptors combined), long-wavelength stimulation (LWS), medium wavelength stimulation (MWS), short wavelength stimulation (SWS), and UV wavelength stimulation (UVWS). Because these five variables were highly inter-correlated, we used principal component analysis (PCA) to identify independent axes of color variation (color PC1, color PC2, and color PC3—see results) that best describe differences among individuals.

Genotyping

Double digest restriction-associated DNA (RAD) sequencing was employed to genotype individuals at a reduced set of SNPs following the protocol described in Peterson et al. (2012).

To prepare libraries, we extracted genomic DNA from 996 *A. carolinensis* individuals using an AutoGenprep 965 instrument and kit. We digested 100 ng of DNA per sample simultaneously with two restriction enzymes: high fidelity EcoRI and Sbf1 (5 units each per sample, New England Biolabs). Following digestion, we cleaned up each sample with AMPure XP beads (Beckman Coulter Genomics) according to the AMPure protocol and quantified the cleaned DNA using a spectrophotometer plate reader (SpectraMax Gemini XS Plate Reader). We then

ligated the digested product to barcoded P1 adapters and biotinylated P2 adapters in a 40 µl reaction with 1 µl of T4 DNA ligase (New England Biolabs). We pooled ligations from 48 samples (determined by the number of unique barcoded P1 adapters we had available) and cleaned up the reactions with two rounds of AMPure XP bead purifications; in the first round, we purified the pooled samples in 6 reactions, and then pooled the eluants from these in one final purification, eluting in 30 µl. We loaded the 30 µl pooled samples onto a 2% Pippin Prep cassette (Sage Science) and selected fragments of mean size 300 ± 35 bp. To enhance sequencing efficiency, we removed P1-P1 ended fragments with streptavidin-coupled Dynabeads® (Life Technologies). We used 5 µl of the eluted Dynabead product as template for 5 replicate PCR amplifications using Phusion, following Finnizyme kit directions (Thermo Scientific). We added a unique index to each library with 24 different reverse PCR primers, and ran each PCR for 12-16 cycles depending on the amount of starting material. We pooled replicate PCR products and cleaned them with AMPure XP beads, and pooled these in turn by index in equimolar ratios and sequenced 1 x 50 bp reads on an Illumina HiSeq2000 instrument.

Following sequencing, we de-multiplexed reads by index and individual barcode. We mapped individual reads to the repeat masked AnoCar2.0 *A. carolinensis* genome (GCA_000090745.2, Ensembl release 70) with BWA (Li & Durbin 2009). To ensure that only high quality reads were used to call genotypes, we merged individual alignments and filtered on two cluster-level metrics: we defined clusters as regions containing overlapping reads, and kept only clusters with 100 - 20,000 reads that had an average mapping quality of 30 or greater. We called variants for this subset of clusters with the Genome Analysis Toolkit's UnifiedGenotyper (McKenna et al. 2010, DePristo et al. 2011, Van der Auwera et al. 2013). We filtered the resulting variants on both site and individual level metrics, keeping bi-alellic SNPs with a quality

score of 20 or more present in 100 or more individuals and requiring each individual to have a minimum depth of 4 and a genotype quality of at least 12. We also tested each site for Hardy-Weinberg equilibrium, and eliminated sites that were not at equilibrium to conform to the assumptions of our parentage analyses. Our resulting dataset for parentage analysis included 90 SNPs.

Parentage Analysis

We assigned hatchlings to mothers and fathers using Cervus v3.0.3 (Marshall et al., 1998; Slate et al., 2000; Kalinowski et al., 2007). Cervus reports the LOD score (the log of the ratio of the likelihood that a parent is the true parent to the likelihood that it is not the true parent) for each parent-offspring pair based on weighted likelihood of shared ancestry for each marker. The program also calculates the delta value, or the difference between the LOD score of the true pair and the next most likely pair for each of the simulated offspring. The probability distribution of delta scores depends on the size of the parent population and the allele frequencies in the actual dataset, therefore we simulated a dataset of parents and offspring with the same properties as our actual population and used the distribution of delta scores from the simulation to select a delta score cutoff of 80% likelihood that a particular pair (parent-offspring) or trio (mother, father, and offspring reflected the true relationship among individuals. For the simulated dataset, we first created 100,000 simulated offspring for the situation where neither parent of an individual is known, but the sexes of potential parents are known, using the allele frequencies calculated from the 90 SNPs in our dataset. The size of our estimated parent pool was 150 potential mothers and 150 potential fathers, similar to the number of adults from our field population, with a conservative estimated sampling rate of 90% based on our field estimated sampling rate of 95%. We used an estimated typing error rate of 0.3%, based on the 0.14% sequencing error rate from

our Illumina HiSeq flowcell, increased due to the possibility of errors introduced during PCR. We re-ran these simulations using a variety of estimates of sampling, typing error, and delta cutoff values. Results were similar in all cases, but we report significance using only the parameters above because they represent a balance between the need for a large sample size with conservatism.

For the parentage analysis of our field samples, we excluded any individuals for which we had fewer than 25 SNPs genotyped. Our final pool of animals included 141 potential mothers, 132 potential fathers, and 675 offspring. Cervus returned delta values for the top mother-father-offspring trio for each offspring in the analysis, as well as the delta values for the parent-offspring pairs within the trio. If the delta value for the most likely trio exceeded the 80% probability threshold from the simulation, we accepted that trio as a true relationship. If the trio did not exceed this threshold, we looked to see if either parent was strongly suggested by the data. If one parent was strongly supported, that is, if the delta value for that parent-offspring pair had a delta value that exceeded the 80% threshold, we accepted that parent as a true parent and left the second parent unassigned. Otherwise, we left both parents unassigned. We then took the total number of offspring assigned to a particular adult as our estimate of that animal's reproductive success (RS). We also assigned a minimum number of mates to each animal based on how many unique members of the opposite sex that animal was recorded producing offspring with.

Data Analysis

Correlates of dewlap color:

To determine if dewlap size and color are correlated with male size, condition, or parasite load, we calculated the linear correlation between dewlap variables (dewlap size, color PC1,

color PC2, and color PC3), and SVL and condition. We also tested whether any of these variables differed between males with and without visible ectoparasites using a Student's t-test. Unless otherwise specified, all statistics were calculated in SPSS v21 (IBM, 2012).

Morphology as a predictor of reproductive success:

We estimated linear selection on dewlap size and color for males using number of offspring as an estimate of fitness. We limited our analysis to males that were repeatedly observed in the study area ("residents") because we were relatively certain that these males were permanent members of our study population. Males for which we conducted fewer than three behavioral observations over the course of the field season ("non-residents") were excluded, because these males may have been itinerants who were present in the population only briefly, or they may have had territories that were largely outside of the study area and therefore their offspring are likely to have been under-sampled. These males are discussed explicitly below.

We considered the following traits as potential predictors of fitness: relative dewlap size, color PC1, color PC2, color PC3 , SVL, and condition. Prior to analysis, all traits were normalized to a mean of 0 and a standard deviation of 1 so that model coefficients were scaled equally for all variables and could be interpreted as standard selection gradients (Lande and Arnold, 1983).

Generalized linear models (GLMs) were used to calculate linear (β) and non-linear (γ) selection gradients on all morphological traits (Lande and Arnold, 1983; Brodie et al., 1995). We used a negative binomial error distribution with a negative binomial link function to calculate selection gradients because this best matched the distribution of our response variable (Scaled Pearson Chi-square test: $X^2 = 48.6$, df = 42, p = 0.22; Kruuk et al., 2002; Robinson et al., 2006).

We tested for collinearity among predictor variables by examining the variance inflation factors (VIFs), all of which were less than 2 in the full model.

We calculated selection gradients in two steps (hereafter referred to as "two-step GLM"): first, linear selection gradients were determined from a model including only linear predictors. Second, non-linear selection gradients were determined from a full model including both linear and non-linear terms (Lande and Arnold, 1983). Because GLMs underestimate quadratic selection terms, both the gradients and standard errors for these terms were doubled (Stinchcombe et al., 2008).

Home range as a predictor of reproductive success:

As above, we calculated linear and quadratic selection gradients on corrected home range size and two PCA-derived estimates of territory quality using a two-step GLM with a negative binomial error structure and a negative binomial link function. All predictor variables were scaled to a mean of 0 and a standard deviation of 1, as above.

Morphology as a predictor of home range:

If home range size or quality has a strong effect on fitness, then morphology may influence fitness primarily by influencing the outcome of territorial behavior. To investigate this possibility, we examined whether morphology (SVL, condition, residual dewlap size, and dewlap color variables) could predict home range size and quality using linear regression. We corrected all predictor variables to a mean of 0 and a standard deviation of 1 so that regression coefficients were comparable among predictor variables.

Resident vs. non-resident males:

Finally, we compared the morphological features of resident and non-resident males (as determined by number of observations, above) to determine if these males differ predictably in

their morphology in a way that could suggest alternative reproductive strategies. We used a Student's t-test of independent samples to test for a difference between SVL, condition, residual dewlap size, and color PC1, color PC2, and color PC3. We also tested whether resident males had more offspring, on average than non-residents.

Results

Field data

Over our four-month field season, we collected a total of 147 adult females, 137 adult males, and 902 hatchlings of *A. carolinensis*.

Behavioral observations

We estimated home ranges for 56 males that were determined to be residents within the study area. An average of 11 observation points were used to calculate the home range of each male (range: 3 to 32 observation points per male), and the average male territory size was 17.7 m^2. We also estimated that resident males had an average of 2.7 male neighbors and 2.6 female neighbors within 5 m of their home range centroid. We estimated home range quality using two axes of variation derived from a principal components analysis of the number of male and female neighbors for each male (Table 2.1): territory PC1 accounts for 70.6% of the variation, and is positively correlated with both male and female neighbors, reflecting how "crowded" a particular area was. Territory PC2, which accounts for the remaining 29.4% of variation, is positively correlated with male neighbors and negatively correlated with female neighbors (Table 2.1). Neither aspect of home range quality is correlated with home range size (territory PC1: $r = 0.15$, $p = 0.28$; territory PC2: $r = 0.12$, $p = 0.39$).

The average distance between mothers and fathers identified by parentage analysis was 33 ± 22 m (Figure 2.2, 2.3). The average distance between any male and female drawn at random from the population was 41 ± 20 m (Figure 2.2).

Table 2.1. Component loadings and variance explained by PCA-derived color variables and PCA-derived territory quality variables. See results for interpretation of component loadings.

Color		**PC1 (brightness)**	**PC2 (hue)**	**PC3 (chroma)**
Eigenvalue		4.35	0.50	0.13
% variance		87.07	9.90	2.66
Loading	Brightness	0.99	-0.04	0.03
	UVWS	0.90	-0.39	0.20
	SWS	0.97	-0.17	-0.11
	MWS	0.96	0.10	-0.24
	LWS	0.82	0.56	0.15
Territory quality		**PC1 (crowding)**		**PC2 (sex ratio)**
Eigenvalue		1.41		0.59
% variance		70.60		29.40
Loading	Male neighbors	0.84		0.54
	Female neighbors	0.84		-0.54

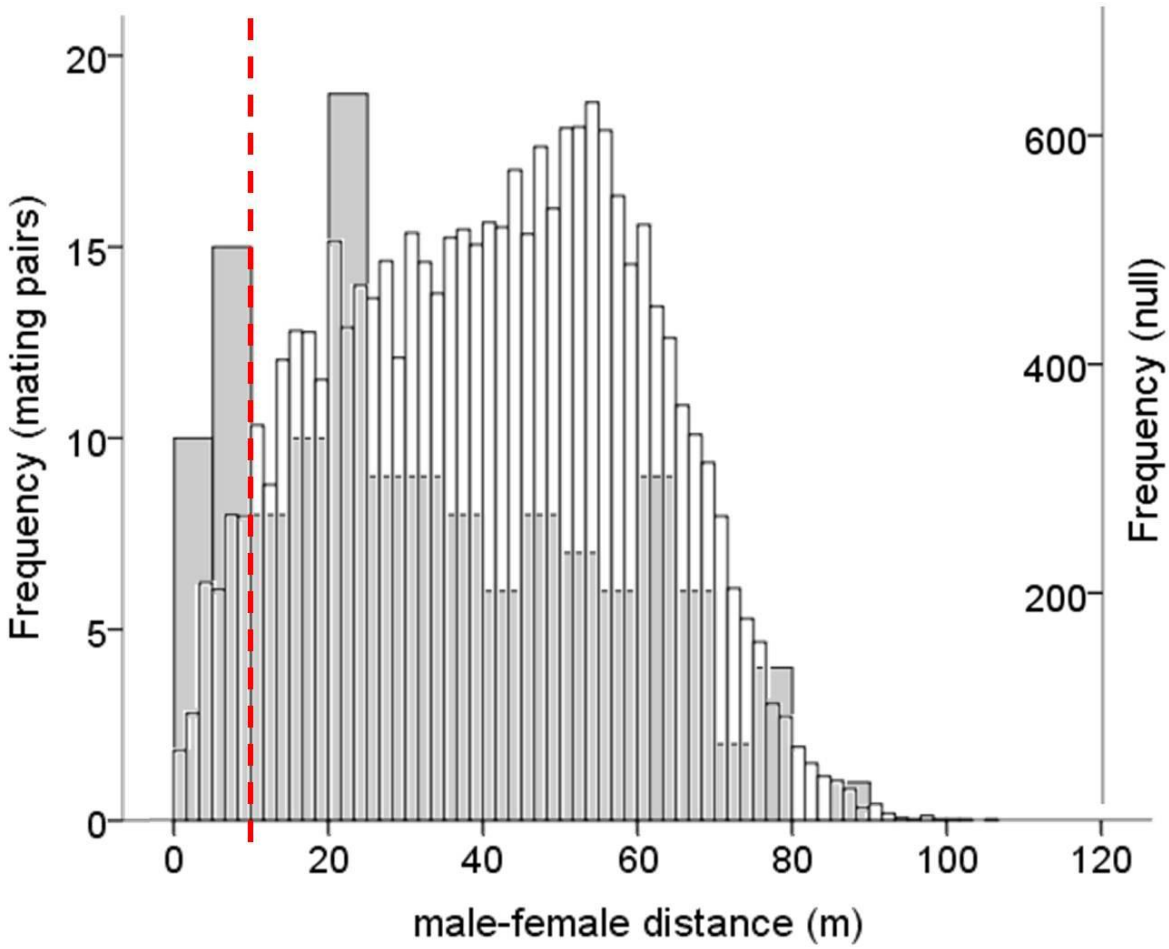

Figure 2.2. The distribution of distances between mothers and fathers identified by parentage analysis (gray) and the null distribution of distances between males and females in the study population (clear). The red dashed line at 9 m indicates the maximum distance between a territory-holding male and a female within that territory.

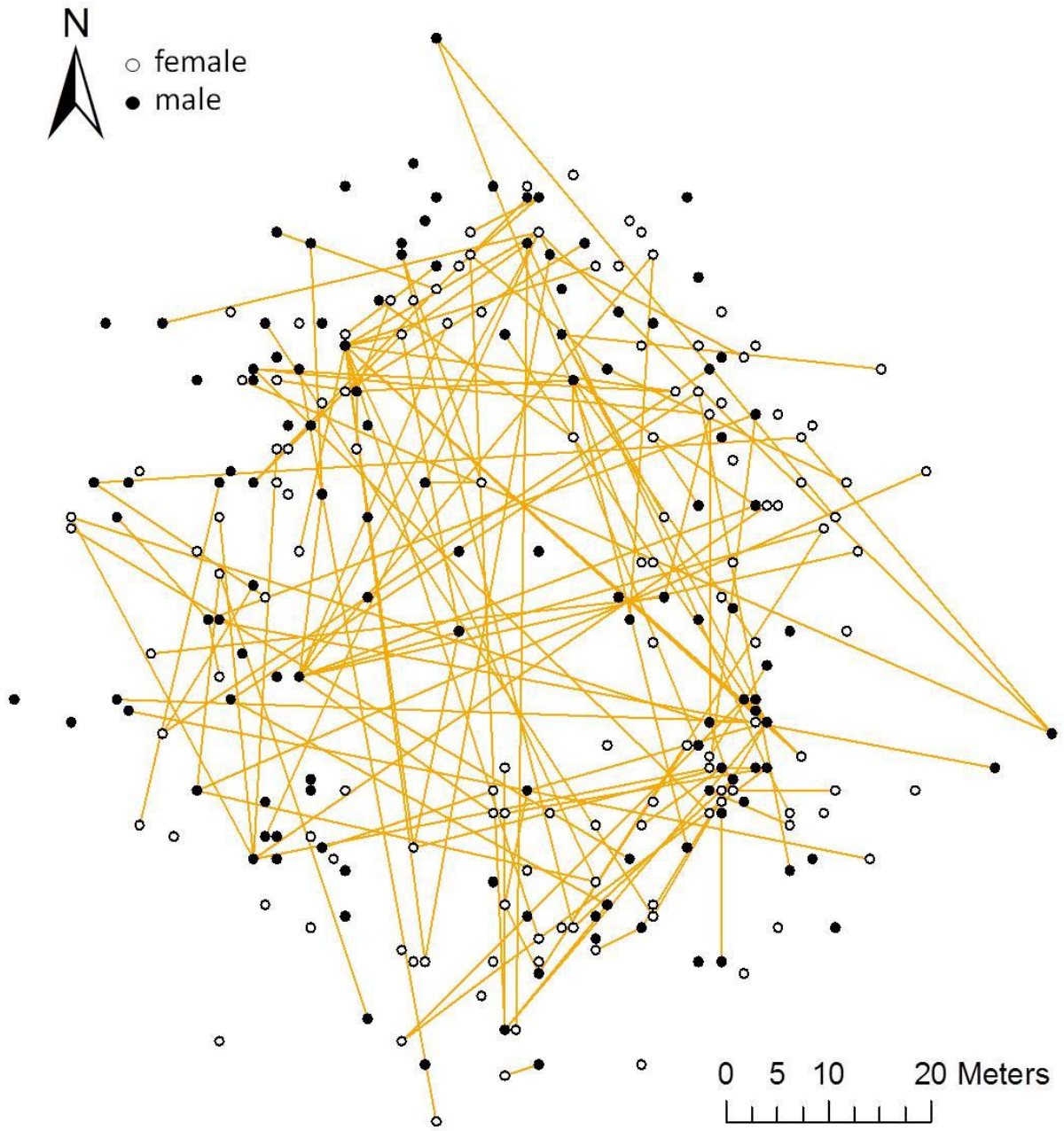

Figure 2.3. Map of adult animals in the study site: females (white) and males (black) with gold lines connecting pairs that produced offspring. Points indicate either the territory centroid (for males with sufficient data) or the coordinates where the animal was first captured.

Dewlap characteristics

Over 99% of the variation in dewlap color was explained by the first three principal component axes (Table 2.1). The first axis of variation (color PC1) accounts for 87.1% of variation in color and loads positively on all variables—a result that is consistent with what is usually referred to as "brightness" (Cuthill et al., 1999; Table 2.1; Figure 2.2, 2.4a). The second axis of variation, which accounts for 9.9% of the total variation in color, is inversely correlated with the UV+blue reflectance of the dewlap and positively correlated with LWS (red) wavelengths. The *A. carolinensis* dewlap has two large spectral peaks, one in the red portion of the spectrum and one in the UV portion of the spectrum, and color PC2 essentially captures a tradeoff between these peaks (Table 2.1; Figure 2.4b). We refer to this axis as "hue" because high values are typical of dewlaps with more red, whereas low values are typical of dewlaps with more UV. The third axis of variation accounts for 2.7% of the total variation in color. Color PC3 is positively correlated with the red and UV peaks in dewlap reflectance and negatively correlated with the blue and green portions of the spectrum such that animals with high values of PC3 have high UV and red peaks with a large valley between them (Table 2.1; Figure 2.4c). We refer to this axis of variation as "chroma" following the conventions of previous studies (Cuthill et al., 1999; Grill and Rush, 2000).

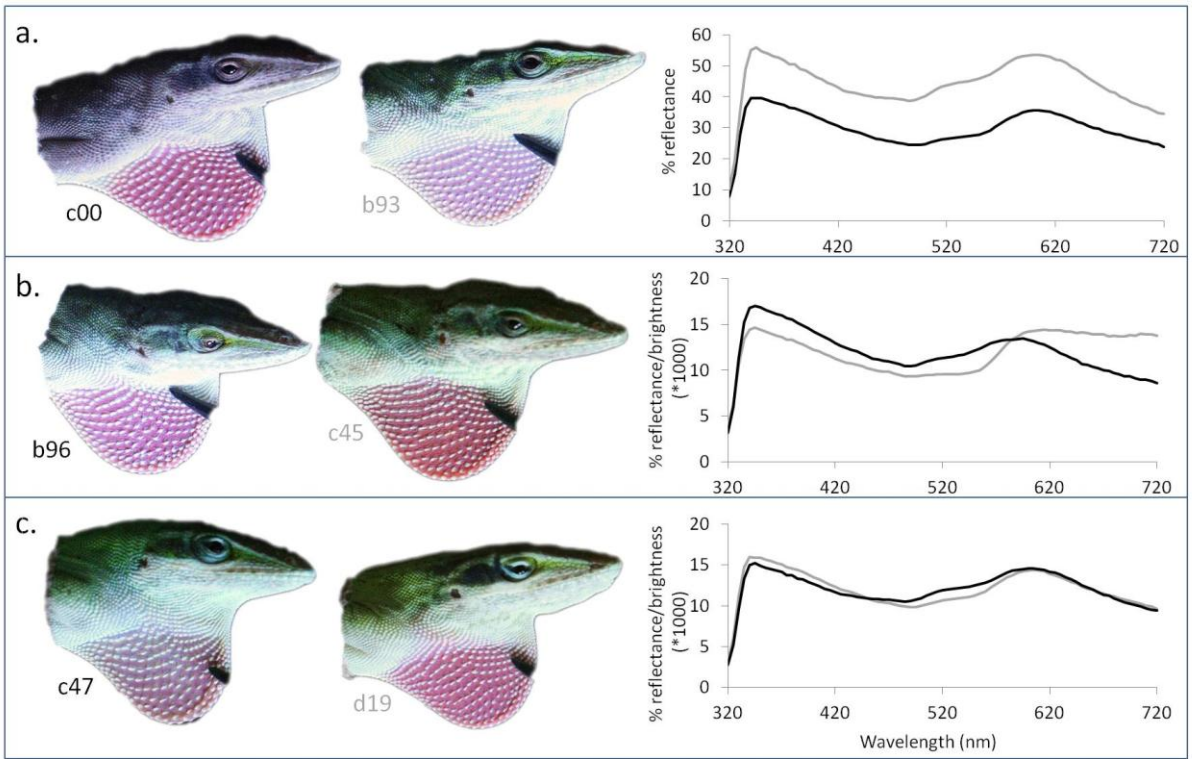

Figure 2.4. Dewlap color variation in male *A. carolinensis* illustrated from photographs taken under standard conditions, and spectral reflectance data. **(a)** Two males showing variation in color PC1: Male c00 (left) shows a low value for PC1, corresponding to a darker dewlap color while b93 (right) shows a high value for PC1, corresponding to a brighter dewlap. **(b)** Two males showing variation in color PC2: Male b96 (left) shows a low value of PC2, corresponding to less red and more UV in the dewlap, while male c45 (right) has a high value of PC2, corresponding to more red and less UV reflectance. **(c)** Two males showing variation in color PC3: male c47 (left) has a low value of PC3, corresponding to more blue and green middle wavelengths and less noticeable red and UV peaks while male d19 (right) shows a high value of PC3, corresponding to higher red and UV peaks with a deeper blue & green valley between them. Spectral reflectance curves shown for a, b, and c show the animal with the lower value (left) in black and the animal with the higher value (right) in grey.

Genotyping and parentage analysis

Of the animals collected in the field, we were able to extract DNA and call SNPs with sufficient resolution for genetic analysis of 141 adult females, 132 adult males, and 675 offspring. Of the 90 SNPs we identified, sequence data were available at an average of 89% of sites for each individual. Using an 80% likelihood cutoff to assign parentage to individual offspring, we were able to assign mothers to 47% of the offspring in our sample, fathers to 53%, and both parents to 19%. On average, females were assigned 1.7 ± 1.8 offspring (mean and standard deviation) and males were assigned 1.8 ± 3.0 offspring. If non-resident males are excluded, the average number of offspring per male is 2.4 ± 4.0. Females that mated had an average minimum number of mates 1.4 ± 0.7 (range: 1-3), and males that mated had an average of 1.8 ± 1.6 (range: 1-9) for all males and 2.1 ± 2.1 (range: 1- 9) when only resident males were included.

Data analysis results

Correlates of dewlap color:

Male body size (SVL) was correlated with brighter (color PC1) dewlaps with more red and less UV (color PC2: Table 2.2, Figure 2.5). Males in better condition had darker (color PC1) dewlaps with lower values of color PC3, or chroma. Larger dewlaps had more UV and less red (color PC2) than smaller dewlaps. Males with parasites differed from males without parasites in that they were larger on average, had smaller dewlaps relative to their body size, and had higher chroma (color PC3, Table 2.3).

Table 2.2. The relationship between dewlap size and color with morphology (Pearson's correlation coefficients). Single asterisks denote significance at $p < 0.05$, while double asterisks denote $p < 0.005$.

	Ln(SVL)	Condition	Relative dewlap size
Relative dewlap size	NA	0.17	NA
Color PC1	0.23**	-0.23**	-0.05
Color PC2	0.23*	-0.04	-0.28**
Color PC3	0.13	-0.17*	0.08

Table 2.3. Comparison of morphology between males with and without parasites. Averages plus/minus standard deviation. Levene's test for the equality of variances were non-significant except for lnSVL. T scores reported on the right reflect this. All tests are two-tailed. Parasitized males were, on average, significantly larger than males without parasites, with smaller, less chromatic dewlaps. Single asterisks denote significance at $p < 0.05$, while double asterisks denote $p < 0.005$.

	Parasites absent	Parasites present	t, p
Ln(SVL)	3.91 ±0.10	3.97 ±0.11	-3.07, 0.003**
Condition	0.08 ±0.99	-0.16 ±0.99	1.33, 0.19
Relative dewlap size	0.12 ±1.03	-0.28 ±1.11	2.10, 0.04*
Color PC1	-0.10 ±0.96	1.81 ±1.05	-1.56, 0.12
Color PC2	0.07 ±1.04	-0.12 ±0.93	1.01, 0.32
Color PC3	-0.14 ±0.96	0.25 ±1.03	-2.16, 0.03*

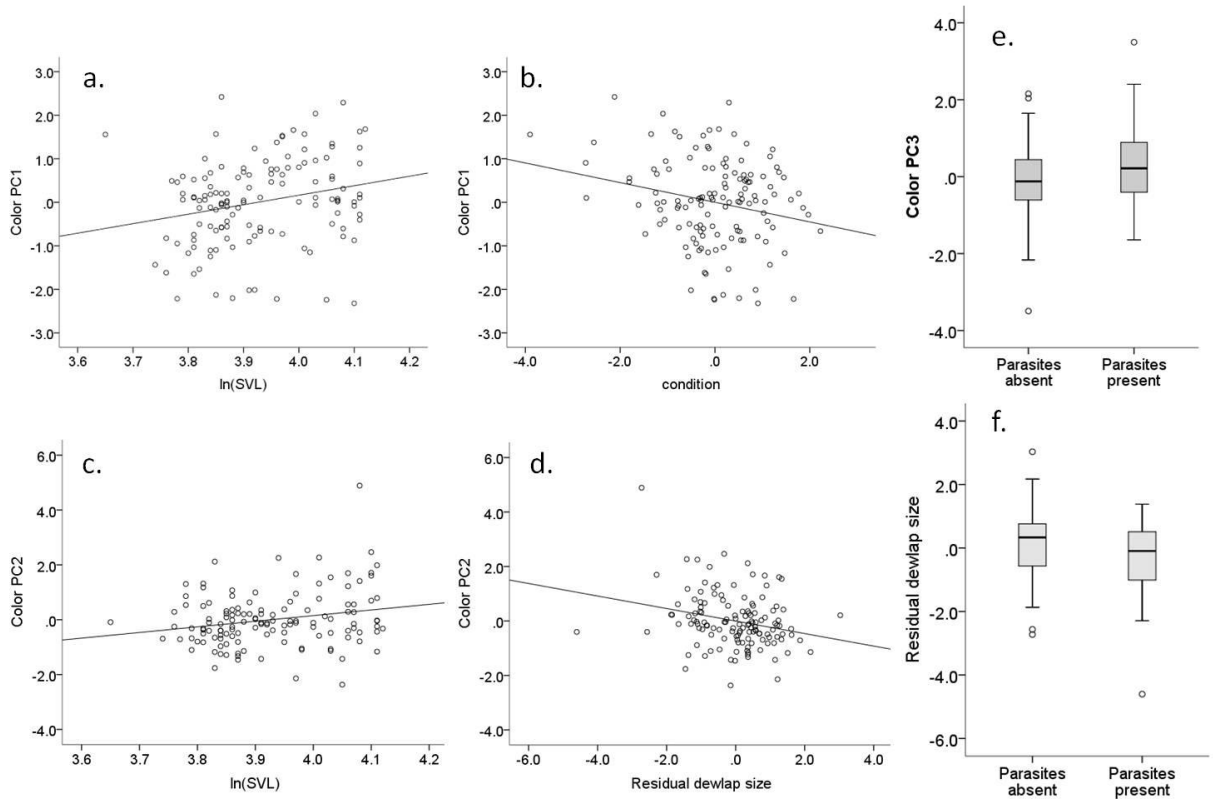

Figure 2.5. (a) Correlation between body size (SVL) and color PC1 for male *A. carolinensis* (r = 0.23, p = 0.01). Larger males tend to have brighter dewlaps. **(b)** Correlation between condition and color PC1 (r = -0.23, p = 0.01). Males in better condition tend to have less bright dewlaps. **(c)** Correlation between body size and color PC2 (r = 0.23, p = 0.01). Larger animals tend to have more red and less UV coloration in their dewlap. **(d)** Correlation between dewlap size and PC2 (r = -0.28, p = 0.001). Males with larger dewlaps have more UV and less red in their dewlaps. **(e)** Difference between males with and without parasites in color PC3 (t = -2.16, p = 0.03). Males with parasites tend to have higher chroma than males without. **(f)** Difference between males with and without parasites in residual dewlap size (r = 2.10, p = 0.04). Males with parasites tend to have smaller dewlaps than males without.

Morphology as a predictor of male reproductive success:

When total male reproductive success is used as an estimate of fitness, we find significant evidence of selection on color PC2 and PC3 (Table 2.4, Figure 2.6a,b; PC2 $\beta = -0.15 \pm 0.07$; PC3 $\beta = -0.11 \pm 0.05$), indicating that males with less red, and more UV, blue, and green reflectance in their dewlaps were more successful at reproducing. Body size (lnSVL), condition, relative dewlap size, and color PC1 did not significantly predict reproductive success.

Home range as a predictor of male reproductive success:

No aspect of home range size or quality was significantly related to reproductive success (Table 2.5).

Morphology as a predictor of home range

In general, morphology was not a strong predictor of home range size or quality (Table 2.6). One exception to this trend was a strong positive relationship between residual dewlap size and home range quality PC2, the ratio of male to female neighbors within a 5 m radius of a male. In other words, males with more male and fewer female neighbors had larger dewlaps relative to their body size.

Table 2.4. Selection on morphology: Linear (β) and quadratic (γ) selection gradients ± standard error for morphological predictors. Single asterisks denote significance at $p < 0.05$, while double asterisks denote $p < 0.005$.

Reproductive success	**β**	**γ**
Ln(SVL)	-0.03 ± 0.06	-5.38 ±6.14
Condition	0.00 ±0.04	-0.07 ±0.08
Dewlap size (residual)	-0.02 ±0.04	-0.03 ±0.05
Color PC1	-0.07 ±0.04	-0.01 ±0.08
Color PC2	-0.15 ±0.07*	0.15 ±0.11
Color PC3	-0.11 ±0.05*	0.02 ±0.07

Table 2.5. Selection on home range parameters. Same procedure as above. Single asterisks denote significance at $p < 0.05$, while double asterisks denote $p < 0.005$.

Reproductive success	**β**	**γ**
Home range area	0.04 ±0.05	-0.04 ±0.18
Territory quality PC1	-0.05 ±0.06	0.04 ±0.14
Territory quality PC2	-0.04 ±0.05	-0.06 ±0.12

Table 2.6. Morphological variables as predictors of home range size and quality. Linear regression slope and error. Single asterisks denote significance at $p < 0.05$, while double asterisks denote $p < 0.005$.

	Home range area	Territory quality PC1	Territory quality PC2
lnSVL	-0.28 ±0.17	-0.18 ±0.15	0.04 ±0.15
Condition	-0.06 ±0.14	-0.07 ±0.15	-0.08 ±0.15
Dewlap size (residual)	0.01 ±0.16	-0.24 ±0.15	0.46 ±0.15**
Color PC1	0.16 ±0.16	-0.17 ±0.15	0.21 ±0.14
Color PC2	-0.06 ±0.16	-0.04 ±0.15	0.02 ±0.14
Color PC3	0.04 ±0.16	-0.19 ±0.15	0.03 ±0.15

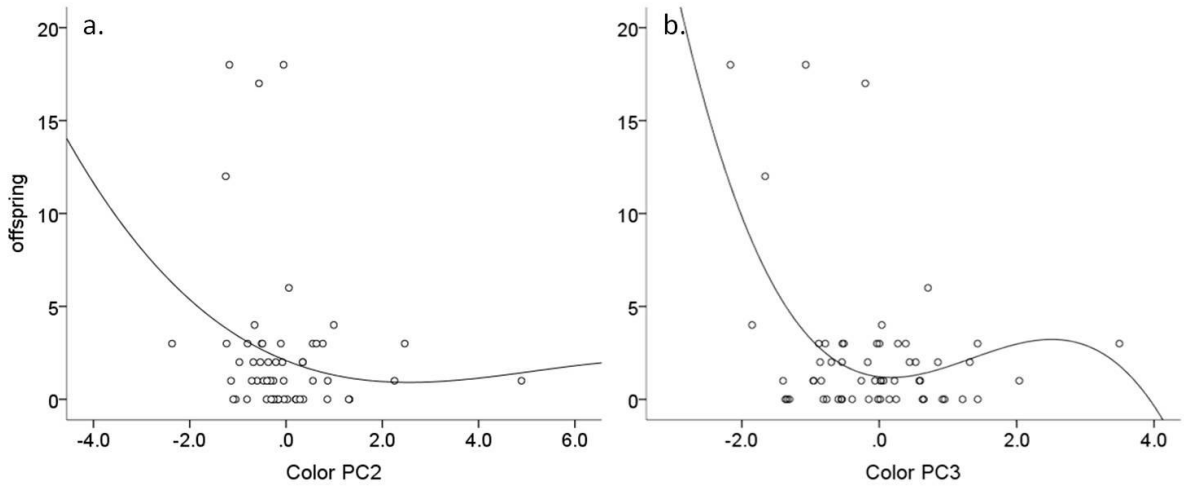

Figure 2.6. Selection surfaces on aspects of dewlap color under selection. **(a)** The relationship between dewlap hue (color PC2) and reproductive success. **(b)** The relationship between chroma (color PC3) and reproductive success. Solid lines represent best-fit cubic spline.

Resident vs. non-resident males

Non-resident males were slightly, but significantly, smaller on average than resident males and were in much worse condition (Table 2.7). They had fewer than half as many offspring as resident males in our study (Table 2.7) though, as noted above, this may be due to undersampling the offspring of males that only passed through the area temporarily. The size and color of the dewlap was similar between non-resident and resident males (Table 2.7).

Table 2.7. Comparison of morphology between resident males (observed on three or more separate occasions) and non-resident males (fewer than three observations). Averages ± standard deviation. Levene's test for the equality of variances were non-significant except for number of offspring. T scores reported on the right reflect this. All tests are two-tailed. Resident males were, on average, significantly larger than non-residents and in significantly better condition. Single asterisks denote significance at $p < 0.05$, while double asterisks denote $p < 0.005$.

	Non-resident average	Resident average	T, p
Ln(SVL)	3.91 ± 0.09	3.95 ± 0.12	2.43, 0.02*
Condition	-0.26 ±1.09	0.30 ±0.80	-3.37, 0.015*
Relative dewlap size	-0.04 ±1.16	0.06 ±0.94	-0.59, 0.55
Color PC1	0.13 ±1.05	-0.13 ±0.92	1.50, 0.14
Color PC2	0.00 ±0.95	0.00 ±1.08	-0.02, 0.99
Color PC3	0.11 ±0.99	-0.12 ±1.02	1.33, 0.19
Offspring	1.34 ±1.5	2.35 ±4.03	-1.84, 0.07

Discussion

Over the past decade, our understanding of social behavior and sexual selection in birds has undergone a dramatic revision as molecular data have been combined with field observations to paint a drastically different picture of avian mating systems (Hughes, 1998; Petrie and Kempenaers, 1998; Griffiths et al., 2002). Among the most surprising discoveries from genetic analyses of parentage was the finding that extra-pair copulations are very common, even in species in which extensive behavioral studies had long suggested genetic monogamy (Griffiths et al., 2002). One consequence of this finding has been a broad re-assessment of the relative importance of intra- and intersexual selection in these species. Specifically, current views suggest that there is far more latitude for female mate choice in many birds, females may use different criteria to choose social partners and genetic fathers, and both male competition and female choice need not end at copulation (Hughes, 1998).

The current view of lizard social behavior, as in birds before the widespread use of molecular techniques, is primarily based on observational studies of social interactions in the lab and the field, leaving open the possibility that equally interesting discoveries await. This view suggests that male lizards often gain access to mates by defending territories in which they secure exclusive mating access to females (Stamps, 1977; 1983; West-Eberhard, 1983; Tokarz, 1995). The predictions of resource-defense polygyny, as this arrangement is commonly called, include multiple mates and high reproductive success for males holding large or high quality territories, a single mate (the territory holder) for females, and lack of access to mates for males that do not defend territories with females. From the few molecular studies that have already been conducted, it is clear that some of these predictions are not supported. Lizards, like birds, mate multiply more often than observational data alone would suggest (reviewed in Uller and Olsson,

2008), and in some territorial species males can succeed using alternative mating strategies that do not include territorial defense (Sinervo and Lively, 1996; Zamudio and Sinervo, 2003). The data in this study confirm that our current understanding of lizard social behavior and evolution can be fundamentally altered by collecting molecular parentage data, even in a species that has been the focus of extensive behavioral and evolutionary research.

Both field and laboratory work with the model lizard *A. carolinensis* have played an important role in advancing our understanding of sexual selection in lizards (Stamps, 1977; 1983; West-Eberhard, 1983; Tokarz, 1995). The mating system of this species has long been considered a classic example of resource-defense polygyny, in which a male's reproductive success depends on his ability to defend a territory from other males (Ruby, 1984; Nunez et al., 1997; Jenssen and Nunez, 2998; Jenssen et al., 2001). The dewlap is presumed to affect male fitness by influencing the outcome of territorial contests between males. We tested two hypotheses deriving from this understanding: (1) size and color of the dewlap are currently under selection, and (2) the primary driver for selection on the dewlap is intrasexual competition for territories that confer access to females.

While we found strong evidence that dewlap color influences reproductive success, there was no evidence that the dewlap influences competition among males for territories or that territory size or quality explains differences in male reproductive success. Our findings, in concert with the observation that members of a mating pair were often located at a large distance from each other, suggest that the classical view of the *Anolis* mating system is incorrect and suggest the possibilities that: (1) female choice is a likely alternative explanation for the relationship between dewlap color and male reproductive success, (2) that the dewlap may serve as an

indicator of male quality, and (3) that alternate male reproductive strategies may also play an important role in this system. We describe each of these possibilities below.

Conclusion 1: dewlap color matters, but not because it influences male competition for territories

Dewlap color is under selection

Our data show clear support for our first hypothesis, that selection is currently acting on dewlap color. Two aspects of dewlap color are correlated with number of offspring in our data. Males with low values of color PC2 had relatively more offspring. These males have less red and more UV/blue in their dewlap (Figure 2.6a). Males with low values of color PC3 also had more offspring. These males have more blue and green and less red and UV (Figure 2.6b). This relationship between color and reproductive success is largely driven by a few highly successful males; only five males fathered more than five offspring in our sample, and these males fathered more than 50% of the offspring in our sample. These males also had very low values of color PC2 and PC3. If dewlap color is heritable, as previous studies suggest (Steffen et al., 2010; Ng and Glor, 2011), these males will have a large influence on the dewlap color of future generations.

Dewlap color is not related to territory, nor is territory related to fitness

Our second hypothesis, that the dewlap is related to fitness via intrasexual competition for territories, was contradicted by several lines of evidence in this study: dewlap color was not related to territory size or quality, and territory size and quality were not related to reproductive success.

The first line of evidence that the dewlap is not a critical component of male territorial competition is the absence of a relationship between any measured aspect of the dewlap or

morphology and any element of territory size or quality. We considered three territory variables: the total two-dimensional area of a male's territory, the total number of neighbors within 5 m of the center of this territory (territory quality PC1) and the ratio of males to females within 5 m of the center of the territory (territory quality PC2). Dewlap color, male size, and male condition were not significantly related to the size or quality of a male's territory.

Only one variable, relative dewlap size, was significantly related to the ratio of males in a territory; males with more male neighbors had larger dewlaps. The significance of this unexpected result is unknown. One possibility could be that dewlap size increases as a plastic response to the presence of other males. Previous studies have found that dewlap size is an indicator of bite force (*A. carolinensis*: Henningsen and Irschick, 2012; *A. lineatopus* and *A. grahami*: Vanhooydonck et al., 2005), but that dewlap size can change dramatically between seasons (Irschick et al., 2006), and does not change in response to dietary restriction (Lailvaux et al., 2012).

Perhaps the bigger surprise in our data is the absence of a relationship between male body size and territory size or quality. Several studies in anoles have found that larger males have larger territories. For example, Trivers found that male body size and territory size were correlated in *A. garmani*. Schoener and Schoener (1982) characterized territorial behavior in four species of *Anolis* from several sites throughout the Bahamas and found that there was a positive relationship between male body size and territory size for *A. sagrei* and *A. smaragdinus* (a close relative of *A. carolinensis* that was once considered the same species), though this relationship was not found in two other species, *A. distichus* and *A. angusticeps*. In *A. carolinensis*, Ruby (1984) found that large males had territories that overlapped with more females than smaller males, and were observed mating more often. Jenssen and Nunez (1998) also found that larger

male *A. carolinensis* had larger territories containing more females. However, another study of *A. carolinensis* found that larger males did not have larger territories or more females within their territories than smaller males (Jenssen et al., 1995).

Although body size and territory size are correlated in several of these studies, this relationship is not universal among species, or even within *A. carolinensis*. One possible explanation for this variation could be differences in methods among studies. Territory sizes have been estimated over vastly different temporal scales, from several days to several months. Shorter studies might underestimate male territory size if males infrequently use some portions of their territory, while longer studies could include changes in body size and shifts in position over time. Another possibility is that the relationship between body size and territory is flexible and depends on the environmental context of the population. For example, in captivity anoles kept at high densities will form dominance hierarchies, while this has never been observed in the field (Greenberg and Crews, 1990; Chizsar et al., 1993).

We believe that our data offer an important perspective on the relationship between territory size and fitness because we were able to estimate reproductive success directly rather than relying on observations of copulations. However, potential shortcomings in our methodology for estimating territory size could also have influenced our results. Our behavioral observations were conducted at irregular intervals, making it difficult to determine whether males shifted their territories during the study period. Ideally, we also would have conducted far more observations of each male during this study.

Another possible explanation for the discrepancy between our data and previous studies could be that our population may have occurred in an atypical environment and this might have changed the relationship between body size and territory size and quality. Our average territory

size, 17.7m^2, was smaller than the 32-44m^2 estimated by Gordon (1950) or the 65.9m^2 estimated by Schoener and Schoener (1982) for a closely related species. Our population occurred in a riparian habitat with abundant prey, and this oasis may have influenced the social behavior of the species by increasing population density, similar to levels observed in captivity (Greenberg, 1990).

Future studies could benefit from more intensive and prolonged spatial and temporal observations of male and female territories, across a range of habitats, to resolve the conditions under which territory size and body size are related.

If not for male territorial competition, why is dewlap color under selection?

We find evidence that dewlap color is related to reproductive success, but our data do not support the classical view that male competition for territories is driving this relationship. A possible alternative is that females are exercising far more choice than has previously been recognized in this genus. Female preferences for particular dewlap colors, combined with the ability to view and assess a large number of males in their general vicinity and find opportunities to mate with males even when these males have home ranges several meters away, could explain both our selection results and the spatial patterns in mating that we find. Dewlap color could be especially important at attracting females from a distance, as Sigmund's (1983) data suggested.

Previous studies have hinted that females may choose mates based in part on dewlap color (discussed in the introduction) and we believe this is the most likely explanation for the patterns we observe. However there are other possible explanations for our data. Dewlap color may influence the outcome of male competition for something other than territory, such as retreat sites or areas with an abundance of prey. This may account for the relationship between fitness and dewlap color if males with a particular dewlap color survive longer and gain more mating

opportunities through their extended lifespan. Alternatively, dewlap color may be correlated with some aspect of male fecundity that influences the outcome of post-copulatory sexual selection. For example, hypothetically, males with lower values of color PC2 and PC3 may have more viable sperm, especially if dewlap color is related to condition in some way.

Another possibility is that males pursue alternative reproductive strategies. Passek (2002) found that small males may reside within the territories of larger males and may account for over 20% of the offspring of females within these territories. Likewise, Lailvaux et al. (2004) found two male morphs; large males with robust heads for fighting and smaller, gracile males that are adept at locomotion. It is currently unclear if males pursue both strategies within their lifespan depending on their body size or if males can use only one strategy. Males in our study did not exhibit a bimodal distribution of body sizes as might be expected if two discrete strategies exist in the population; nonetheless, males may use alternative tactics. If males are utilizing different strategies to gain mating access to females, this could obscure the relationship between territory size and reproductive success in males that pursue a territorial strategy because we did not separate males by strategy for our analysis.

Spatial proximity does not predict mating relationships

An additional surprise in our data was that members of mating pairs were often widely separated in space and that males and females often mated multiply. If males achieved reproductive success by defending exclusive mating access to sedentary females within an area, as previous authors have suggested (Stamps, 1977; 1983; West-Eberhard, 1983; Tokarz, 1995), then most offspring should be produced by males and females found in close proximity to each other—on the range of 5m (Gordon, 1956; Jenssen et al., 1995). Males would be expected to have multiple mates but females would not. Our data are not consistent with these expectations.

Previous studies have suggested that multiple paternity may not be uncommon in lizards (Uller and Olsson, 2008), including anoles (Passek, 2002), and our results confirm that female *A. carolinensis* often mate with more than one male. However, the wide distance between mating pairs was wholly unexpected based on previous observations of behavior. We find that the average distance between males and females that mated successfully is quite large, far beyond the distance expected between males and females sharing a territory (Figure 2.2), or even animals from adjacent territories. In fact, males and females from opposite sides of the study site mated relatively frequently (Figure 2.3), often traversing distances over 60 m. For this to occur, either the male or female (or both) left its territory at some point, or they mated before establishing territories and used stored sperm (Fox, 1963). The habitat at this study site was relatively open, so it is possible for a female to view a male displaying from a long distance. We did not observe animals moving these distances, nor have previous researchers, so we cannot speculate whether males or females left their territories to copulate, or when they made these movements.

One hint as to how these extraterritorial matings occurred might be drawn from the literature on birds. In superb fairy wrens, females make pre-dawn forays to the territories of neighboring males seeking extra-pair copulations (Double and Cockburn, 2000). Female forays to male territories and subsequent genetic evidence that females mated with males from these territories have been documented in at least a dozen species (Westneat and Stewart, 2003), although actual observations of copulation during these forays are rare. Female *A. carolinensis* are less conspicuous than males, both due to their coloration and their behavior. Perhaps females in this species are also able to make undetected forays away from their usual territory. Another possibility is that males alternate between territories, or cease to defend their territories on

occasion to wander through the habitat seeking mating opportunities (see further discussion below). Gordon (1956) found that male *A. carolinensis* sometimes disappeared from their territories for several weeks before reappearing and resuming their territorial activities. Where they went during these periods was unclear. Males were observed making small forays outside their territories to copulate in two other species of *Anolis*, *A. garmani* and *A. sagrei* (Trivers, 1976; Tokarz, 1998). Further, female *A. carolinensis* were found to move less frequently that males, moved shorter distances, and inhabited smaller home ranges (Nunez et al., 1997), suggesting that males may be the more likely sex to leave their territories. Another possible behavioral indication that males and females that do not share territories may come into contact, also described by Gordon, was that males sometimes utilized sleep sites that were outside of their defended territory. Perhaps males and females moving to and from their sleep sites have the opportunity to interact even if they are active in different areas during the day. In superb fairy wrens, female forays before dawn were not detected by direct observation, but were discovered only by tracking individual females using radio telemetry (Double and Cockburn, 2000). Our data suggest that a similar approach may be informative with anoles.

Conclusion 2: Dewlap color could indicate parasite infection, size, or condition

Colorful ornaments can be favored by selection for a variety of reasons, including female preferences and species, sex, or individual recognition (Bradbury and Vehrencamp, 1998). Female preferences can arise when ornaments indicate something about male quality (indicator traits), because ornaments mimic other environmental cues that females are alert to (sensory bias), or because innate preferences can be caught in a self-reinforcing runaway cycle (Fisherian runaway selection) (Andersson, 1994).

Previous studies of the information content of the dewlap in anoles have focused on the potential for the dewlap to facilitate species or sex recognition. Experiments on dewlap color have shown that females are mistaken for males when their dewlaps are manipulated to resemble male dewlaps (Greenberg and Noble, 1944). Likewise, when males of one species are painted to resemble males of a second species, they attract far more aggression from males of the second species and less aggression from males of their own species (Losos, 1985).

Our data provide mixed support for a third possibility, that dewlap color is an indicator of male quality. Color PC2, which we found to be a significant predictor of male fitness, is weakly correlated with male body size and dewlap size (Figure 2.5c). Males with lower values of color PC2, those with higher fitness, tended to be smaller but with relatively larger dewlaps than males with high values of color PC2. Color PC3, which was also a significant predictor of male reproductive success, is correlated with condition such that males in better condition had lower values of color PC3 (Figure 2.5d). Males with lower values of color PC3 also had higher fitness. Color PC3 was also higher in males with visible mites (Figure 2.5e). Males infested with mites were also, on average, larger than males without mites and had relatively smaller dewlaps (Figure 2.5f). The brightness of the dewlap, color PC1, is positively correlated with male body size and negatively correlated with male condition. Males with brighter dewlaps are therefore likely to be larger and in worse condition than males with darker dewlaps. However, we did not find evidence for selection on color PC1, suggesting that this is not an aspect of the signal that is important to mating success. Laboratory tests of female preference should be conducted to demonstrate female preferences conclusively and separate whether ornaments are truly operating as indicators of male quality. If females exercised a preference for low values of color PC2 and PC3, as our selection results suggest they may, they would tend to mate with smaller males in

good condition, and with a lower chance of carrying mites. Interestingly, we did not find evidence for direct selection on male body size, dewlap size, or condition.

The relationships we observed between the dewlap and male size, condition, and parasite load are not very strong, which may be why we did not find evidence for selection on body size or condition. Alternatively, it may be that female preferences for dewlap color evolved under conditions in which the relationship between color and quality was stronger and that the preferences have persisted. Finally, it is possible for female preferences to evolve to favor male traits that are not indicators, such as through sensory biases or Fisherian runaway selection (Andersson, 1994). It can be difficult to distinguish between these alternatives, even with controlled mate preference trials under laboratory conditions. An investigation of the genetic architecture of dewlap color and female mate choice might be able to untangle these possibilities.

Conclusion 3: some males might employ a non-territorial strategy

Many males in this study were observed on the study site on only one or two occasions. These males were excluded from the analysis because they were likely not permanent residents in the study area and thus the total number of their offspring is likely to have been under-sampled by our spatially limited methods. Nonetheless, non-resident or "transient" males accounted for roughly 2/3 of the males observed in the study area over the course of the summer, and fathered about 1/3 of the offspring assigned in this study. Whether these males were observed infrequently due to high mortality, because they were temporarily searching for a vacant territory, because they had territories outside the study site that they had temporarily moved from, or because they represented a persistent itinerant strategy with the bulk of their home range outside the study area is unclear. Our data are not spatially or temporally resolved

enough to determine the origin of these males, the spatial and temporal scope of their wandering, or their ultimate fate.

Previous work on *A. carolinensis* has suggested that transient males are not a unique phenomenon to this population. Out of 56 males observed at two field sites in Louisiana, Gordon (1956) found that only 14 of these males were considered residents (observed on three or more occasions). Even resident males often went missing for several weeks at a time, only to reappear and resume their territorial activities. Gordon suggested the possibility that these males may have dual home ranges, and that males observed on only one or two occasions may have been removed from the population by predation. Another hint that transient male *A. carolinensis* may be widespread come from Passek's (2002) data: of the 16 males observed in this study, all were deemed either territory holders or small covert males coexisting in a larger male's territory, yet 12% of the offspring in this study were fathered by males that were not sampled in the study area. While sperm storage by female *A. carolinensis* makes it impossible to determine if these were the posthumous offspring of previous territory holders or males that were too cautious to capture, another possibility is that these offspring were the result of females mating with males that had passed through the area only briefly at some point. A third potentially relevant observation is that previous observational studies of *A. carolinensis* have found a discrepancy between the sex ratio, which is generally roughly 1:1, and the ratio of females to males within a male's territory, which averages 2-3 females per male (Ruby, 1984; Nunez, 1994; Jenssen et al., 1995). Some of the "missing males" from these studies may be small males covertly inhabiting a larger male's territory (Trivers, 1976; Jenssen and Nunez, 1998; Passek, 2002), but a significant number of males remain unaccounted for. Jenssen et al. (1995) suggest that many males must hold territories that contain no females, yet surely such males would benefit from attempting

forays into areas with females, even if this attracts aggression from a territorial male. In fact, female *A. garmani* were observed copulating with males either intruding from a neighboring territory (2/49 copulations observed) or transient males not observed before or after (1/49; Trivers, 1976). Female *A. sagrei* also mate with males intruding from nearby territories, at least occasionally (Tokarz, 1998).

We are tempted to speculate that transient males represent an alternative reproductive strategy in A. *carolinensis*, although the data from this study are suggestive rather than conclusive. We can imagine three possible scenarios. First, transient males may represent a stable strategy for some males. These males may have a very large undefended home range that overlaps with the defended territories of other males, and they may pass infrequently through each portion of the home range. If this is the case, then male home range size may be bimodally distributed, with some males covering very large areas that they do not defend against other males. A second scenario is that males may adopt a transient strategy when they are too large to live undetected in another male's territory, but too small to wrest an existing territory from an established male. If this is the case, then transient males should be intermediate in size. In their study of life-stage morphs in *A. carolinensis*, Lailvaux et al. (2004) found that males transition from gracile to large-headed at about 65 mm, so this may coincide with a transient stage of transition. Males in our study were smaller than those in Lailvaux et al. (2004), so we cannot make a direct comparison to their data, but resident and transient males in our study were only slightly different in size with substantial overlap between groups. A final possibility is that territorial males may periodically opt to shift their strategy for a period of time and leave their territory to explore the surrounding area. This could provide an opportunity to rest and recuperate from the energetic costs of territorial defense, allow males to assess if there are better

territorial opportunities nearby and build a mental map of the broader area, or provide males with the opportunity to court novel females, for which they have a demonstrated preference (Orrell and Jenssen, 2002). If this is the case, then closely tracking territorial males should reveal alternating periods of site fidelity and roaming.

The suggestion that transient males represent an alternative mating strategy in this species is speculative at this point. Other possibilities, such as high mortality, cannot be ruled out from our data. Nonetheless, we believe that it would be worth the effort to conduct careful radio telemetry studies over relatively long periods of time to investigate this interesting possibility.

Summary

Broadly stated, the results of this study are: (1) The color of the dewlap of male *A. carolinensis* is currently under selection in our study population, (2) dewlap color is not correlated with territory size or quality in males, (3) territory size and quality are not currently under selection, (4) males and females often mate with individuals with whom they do not share a territory, and may traverse relatively large distances to do so, (5) both males and females mate multiply, (6) dewlap color is weakly correlated with body size and condition, as well as the presence of parasites, (7) males in this population were often observed on only one or two occasions.

From these results we conclude: (1) dewlap color is important to fitness, (2) male competition for territories is not as important to fitness as previously believed, and (3) the mating system of this population is not consistent with resource-defense polygyny. We speculate that dewlap color may be involved in female choice, perhaps because it can serve as an indicator of male quality; that males or females may be cryptically moving out of their territories seeking opportunities to mate with animals some distance away; and that some "transient" males may

either temporarily or permanently use a wide-ranging, non-territorial strategy to find mates. We hope that future studies will investigate the possibility of female choice in anoles more closely under controlled conditions, and that more detailed long-term studies of movement patterns will help determine when and how animals mate with partners from distant territories and whether transient males represent an alternative mating strategy.

When molecular data were brought to bear on the study of social behavior in birds, it revolutionized our understanding of mating systems and sexual selection. Our results suggest that similar insights will result from combining molecular and field data to study mating systems in *Anolis*. This study represents a first step towards a more accurate understanding of the mating behavior of an important model species in evolutionary ecology, and a better understanding of the factors that have driven the evolution an impressively diverse ornament, the *Anolis* dewlap.

Chapter 3

Variation in dewlap size, color, and pattern among recently introduced populations of *Anolis sagrei*

Abstract

Groups of species with colorful ornaments are often highly diverse, perhaps in part because ornaments are subject to strong and diverse types of selection. The lizards genus *Anolis* is one such group: among the ~400 species of anoles, the colorful throat fan, or dewlap, comes in a surprising variety of color and patterns. Although several ideas have been proposed to explain this variation, it remains unclear how and why the dewlap has evolved such dramatic diversity. In the present study, we took advantage of the recent widespread anthropogenic introduction of *A. sagrei* to characterize the earliest stages of dewlap divergence among populations and to look for variables that might explain intraspecific dewlap divergence. In contrast to most anole species, which exhibit little intraspecific variation, we found extensive variation among populations in the size, color, and pattern of the dewlap. Only a small fraction of this variation was explained by the genetic relationships among populations, while a large portion of the variation was explained by differences in ecological variation among sites, suggesting that the dewlap is evolving rapidly in response to selection in novel environments. Variation among populations was partially explained by differences in sexual dimorphism, predation rate, anole community complexity, and climatic differences among sites, suggesting several avenues for future research. This study presents some of the first data showing rapid divergence in an ornament in introduced populations of a widespread exotic species.

Key words: ornament evolution, *Anolis sagrei*, exotic species, visual signals, sexual selection, predation, species recognition, sensory drive, speciation

Introduction

Highly diverse groups of animals are often characterized by elaborate, variable ornaments, perhaps in part because ornaments can promote diversification when they serve a species recognition or mate choice function (Barraclough et al., 1995; Price, 1998; Owens et al., 1999; Stuart-Fox and Owens, 2003; Mank, 2007; Ritchie, 2007; Wagner et al., 2012). The neotropical lizard genus *Anolis* is a classic example of a diverse genus characterized by a colorful ornament. The dewlap, a patch of colorful skin on the throat that is stretched over the second elasmobranchial cartilage during a display, shows astonishing variation in size, color, and pattern among species (Williams and Rand, 1977; Losos and Chu, 1998; Nicholson et al., 2007; Harrison and Poe, 2012).

Several hypotheses have been proposed to account for dewlap variation, including sexual selection (Greenberg and Noble, 1944; Fitch and Hillis, 1984; West-Eberhard, 1983; Harrison and Poe, 2012), species recognition (Williams and Rand, 1977; Losos, 1985; Nicholson et al., 2007), predator avoidance (Leal and Rodruiguez-Robles, 1997), and sensory drive (Losos and Chu, 1998; Leal and Fleishman, 2002). The first three concern the receiver of the dewlap signal (conspecifics, congeners, or predators, respectively) while the fourth concerns the efficacy of signal transmission in different environments. Neutral processes like genetic drift, admixture, or founder effects may also cause divergence in dewlap color (Kolbe et al., 2004; 2007).

The first of these hypotheses is that sexual selection drives changes in the size, color, or pattern of the dewlap. Sexual selection could trigger different evolutionary trajectories among

populations for example if females prefer novel signals (Panhuis et al., 2001; Holland and Rice, 1998), or through an interaction between natural and sexual selection (Maan and Seehausen, 2011). When sexual selection is especially strong, populations may diverge more quickly. If traits and preferences coevolve such that individuals will mate assortatively with members of their own populations, sexual selection could promote both speciation and ornament diversity (Maan and Seehausen, 2011). The evidence that female anoles exercise mate choice on the male dewlap is inconclusive (see chapter 1), but the use of the dewlap during courtship suggests mate choice may occur at least under some conditions.

There is relatively strong indirect support for the species recognition hypothesis of dewlap diversity. Within complex communities, the dewlap is often more elaborate than in single-species communities, and there are almost no cases where two species have identical dewlaps (Rand and Williams, 1970; Nicholson et al., 2007). There is also compelling evidence of character displacement in dewlap color (Webster and Burns, 1973; Lambert et al., 2013), and experimental evidence that manipulating dewlap color can interfere with species recognition (Losos, 1985).

Predation may also lead to dewlap differences among populations for a variety of reasons. In anoles, previous research has suggested that anoles both try to avoid the attention of predators (Cantwell and Forrest, 2013; Driessens et al., 2013; Steinberg et al., 2014), and also use the dewlap as a pursuit-deterrence signal (Leal and Rodruiguez-Robles, 1995; 1997; Leal, 1999). One intraspecific comparative study suggested that pursuit-deterrence could be responsible for the evolution of larger dewlaps in populations where a saurian predator, *Leiocephalus carinatus*, was present, presumably because larger dewlaps are more effective at deterring predation

(Vanhooydonck et al., 2009). Predation could lead to variation among populations if the intensity of predation or the identity of predators differs among sites or microhabitats.

Finally, the sensory drive hypothesis proposes that differences among environments can determine the efficacy of a signal, regardless of whether the signal is intended for conspecifics, congeners, or predators (Endler, 1992; Boughman, 2002). For example, in bright, open habitats darker colors may offer more contrast against the background while in dark habitats pale signals may be more conspicuous (Endler, 1993; Fleishman, 1992; 2000).

Variation both within and among species of *Anolis* is suggestive of sensory drive as a force in the evolution of dewlap color. As predicted, species that inhabit dense forest tend to have white or yellow in their dewlaps while species living in open habitats often have darker dewlaps (Fleishman, 1992; 2000). Variation in the dewlap color among populations of *A. cristatellus* is also consistent with sensory drive (Leal and Fleishman, 2004), and similar patterns have been observed in other species (Thorpe, 2002; Thorpe and Stenson, 2003). More recently, Ng. et al. (2012) found that dewlap color in populations of *A. distichus* is correlated with precipitation, temperature, and tree cover and conclude that sensory drive is likely to explain these patterns.

Trait divergence among populations can also result from neutral genetic processes, such as genetic drift or founder effects (Coyne and Orr, 2004). In anoles, there is little evidence to suggest that founder effects have played an important role in diversification, of the dewlap or any other trait, while the relative importance of genetic drift among populations is unknown. Species that frequently colonize new islands (such as *A. carolinensis* and *A. sagrei*) might be expected to show evidence of founder effects, but the classical view is that these populations showed minimal phenotypic divergence (with a few notable exceptions: see Losos, 2009), and the genetic diversity of introduced populations is often higher than that of source populations

(mainly due to admixture; Kolbe et al., 2007). Furthermore, an experimental test of founder effects in anoles did not find any difference in divergence in populations founded with five versus ten individuals (Losos et al., 2001).

Genetic drift, on the other hand, may be more important. Phylogeographic studies of anoles have found substantial genetic differentiation among populations within species, even in the absence of notable phenotypic differentiation (Jackman et al., 2002; Glor et al., 2003; 2004; 2005), which could be a consequence of genetic drift. Whether drift can also explain phenotypic divergence is less clear. In some cases, genetic breaks between populations coincide with morphological differences (e.g. Schneider, 1996; Malhotra and Thorpe, 2000; Thorpe, 2002), but this could result from either selection or drift.

Introduced populations may provide a unique opportunity to test evolutionary hypotheses like these by focusing on the earliest stages of divergence among populations. Introduced populations face sudden and dramatic shifts in their biotic and abiotic environments that are likely to lead to strong selection (Hanfling and Kollmann, 2002; Huey et al., 2005). These "accidental experiments" have allowed evolutionary biologists to document surprisingly rapid evolutionary changes in a variety of morphological and behavioral traits (Huey et al., 2005; Sax et al., 2005; Sax et al., 2007; Prentis et al., 2008; Kolbe et al., 2014). More recently, the novel conditions experienced by introduced populations have been recognized as a potential source of novel evolutionary diversity, and even potentially of speciation (Velland et al., 2007). The evolution of animal ornaments in introduced populations may prove especially interesting, both because we have several well-developed hypotheses to predict how and why ornaments evolve, and because ornament divergence could result in reproductive isolation between distinct populations.

The Cuban brown anole, *Anolis sagrei*, provides an ideal case study for the study of rapid ornament evolution in invasive populations. The *A. sagrei* dewlap is relatively variable, ranging from a solid dull ultraviolet (uv)-yellow to deep brick red, with many dewlap exhibiting uv-yellow on the edge of the dewlap and red in the center, though dewlaps are also occasionally striped with red and uv-yellow (personal observation; see also Vanhooydonck et al., 2009). Among anoles, *A. sagrei* has also been exceptionally successful at invading new areas (Lever, 2003), providing many replicates for evolutionary studies. Finally, for many of these populations, the genetic history of the invasion is also known (Kolbe et a., 2004; 2007).

In the present study, we sought to answer two questions about variation in the size, color, and pattern of the dewlap in introduced populations of *A. sagrei*. First, do populations show significant variation despite their recent origins? Second, is the observed variation correlated with any variables suggested by the five hypotheses discussed above, namely sexual selection, species recognition, predator avoidance, sensory drive, or genetic effects?

To address these questions we gathered data on male and female *A. sagrei* morphology, estimated avian predation rate from attacks on models, and recorded the presence and identity of congeners at sites where the species has been present for less than 150 years. This study provides insight into ornament evolution both because of the large number of populations considered and because it is one of the first studies of which we are aware that looks at the evolution of an ornament in an invasive species.

Materials and Methods

Between August 2008 and December 2013, we collected data on dewlap variation among populations in for male *A. sagrei* at 25 sites in Florida, Texas, Louisiana, and Hawaii in the

United States, as well as Jamaica, the Cayman Islands, and Honduras (Table 3.1, Figure 3.1a). Of these, *A. sagrei* was clearly present as the result of a recent introduction in 19 sites, while its origin was unknown and may have predated European colonization in six cases. We collected a total of 846 adult males and 380 adult females, either by hand or noose. For each animal, we measured the snout-vent length to the nearest 0.1mm using digital calipers.

Table 3.1. Sites and variables included in this study. The letters in column 1 correspond to the letters in Figure 3.1 and in the text.

	Site	Location	Age	Genetic data	N (f,m)	SSD	SD C	Predation	Congeners
a.	Houston	Texas, USA	1985	Yes	23, 26	*1.33*	0.16	0.29	*A. carolinensis*
b.	Corpus Christi	Texas, USA	1986	Yes	19, 26	*1.21*	0.28	0.23	*A. carolinensis*
c.	New Orleans	Louisiana, USA	1990	Yes	47, 43	*1.25*	n/a	0.23	*A. carolinensis*
d.	Oahu	Hawaii, USA	1980	Yes	13, 34	*1.29*	0.19	0.10	*A. carolinensis, A. equestris*
e.	GTM-NERR	Florida, USA	1975	No	49, 37	*1.26*	0.11	n/a	*A. carolinensis*
f.	Gainesville	Florida, USA	1980	Yes	0, 27	*1.37*	n/a	n/a	*A. carolinensis*
g.	Seminole Rest	Florida, USA	1989	Yes	18, 24	*1.30*	0.22	0.17	*A. carolinensis*
h.	Tampa	Florida, USA	1946	Yes	0, 26	*1.28*	n/a	n/a	*A. carolinensis*
i.	Collier-Seminole	Florida, USA	1976	Yes	0, 42	*1.37*	n/a	n/a	*A. carolinensis*
j.	Lizard	Florida, USA	1995	No	9, 17	*1.22*	0.17	0.00	*A. carolinensis*
k.	Yin	Florida, USA	1995	No	12, 22	*1.29*	0.21	0.00	*A. carolinensis*
l.	Ant	Florida, USA	1995	No	15, 21	1.29	0.21	0.10	None
m.	Line of Cedars	Florida, USA	1995	No	12, 10	1.22	0.20	0.10	None
n.	Rockpile	Florida, USA	1995	No	6, 16	*1.34*	0.18	0.10	*A. carolinensis*
o.	Coon	Florida, USA	1995	No	14, 24	1.31	0.22	0.00	None
p.	G43	Florida, USA	1995	No	9, 22	*1.26*	0.20	0.00	*A. carolinensis*

Table 3.1 continued

q.	Utila	Honduras	2005	No	29, 17	*1.29*	0.06	n/a	*A. bicaorum, A. sericeus, A. utilensis*
r.	Ceiba	Honduras	n/a	No	20, 26	*1.20*	n/a	n/a	*A. allisoni*
s.	Swan Island	Honduras	n/a	No	22, 32	1.30	n/a	0.10	None
t.	Little Swan Island	Honduras	n/a	No	10, 19	1.30	n/a	n/a	None
u.	Little Cayman	Cayman Islands	n/a	Yes	0, 22	*n/a*	n/a	n/a	*A. maynardi*
v.	Cayman Brac	Cayman Islands	n/a	Yes	8, 198	1.14	n/a	n/a	None
w.	Grand Cayman	Cayman Islands	1984	Yes	7, 35	*n/a*	n/a	n/a	*A. conspersus*
x.	Bluefields	Jamaica	n/a	Yes	16, 42	*1.20*	0.53	0.17	*A. grahami, A. lineatopus, A. opalinus, A. garmani, A. valencienni*
y.	Robin's Bay	Jamaica	1960	Yes	12, 28	*1.17*	0.33	0.23	*A. grahami, A. lineatopus, A. opalinus, A. garmani, A. valencienni*

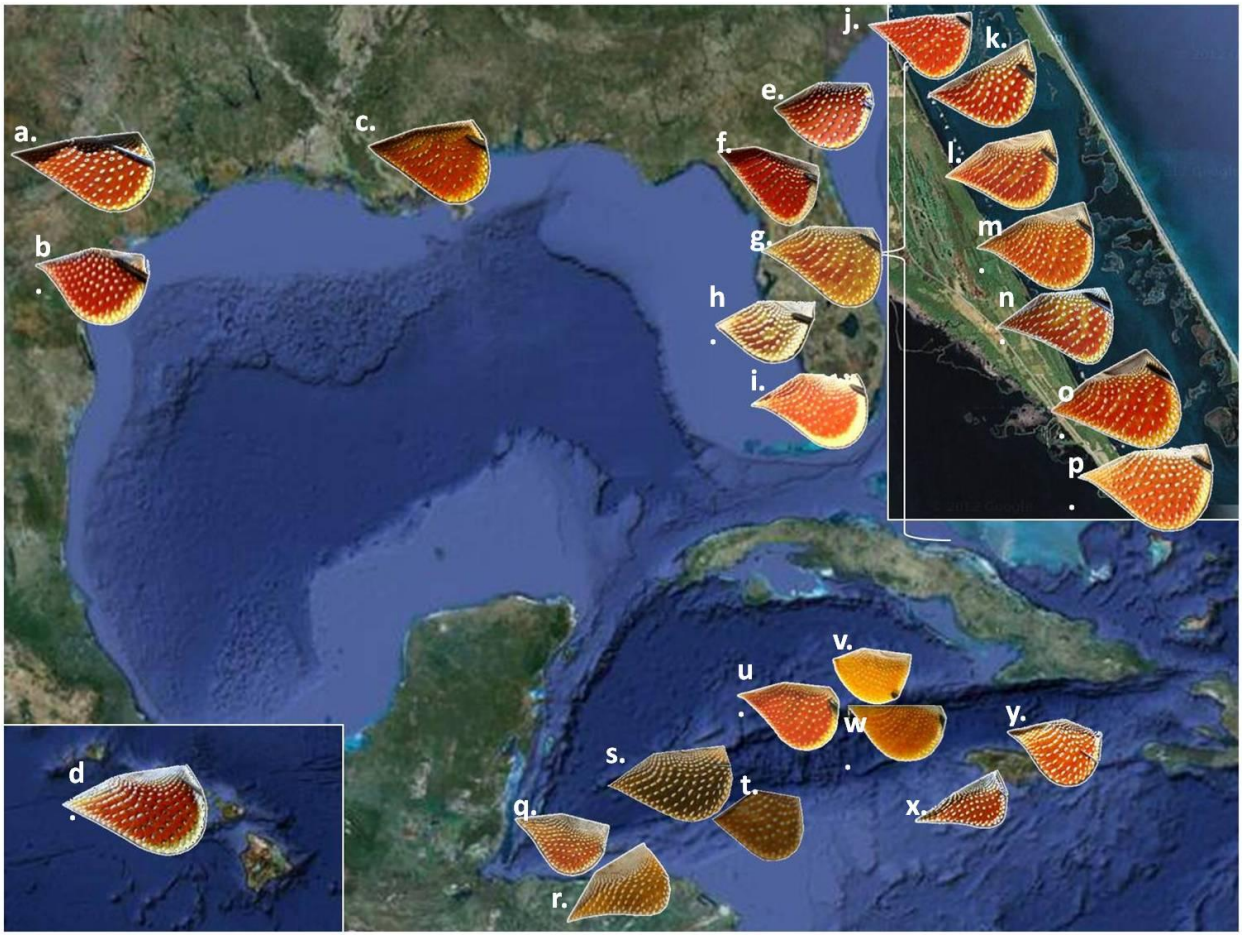

Figure 3.1 (a). Representative dewlaps from 25 sites in Texas, Louisiana, Hawaii, Florida, the Cayman Islands, Jamaica, and Honduras. Dewlap photos were color-normalized to the greatest extent possible, to minimize apparent differences caused by variation in the light conditions where photos were taken. Each photo was also size adjusted so that the relative size of dewlaps from different sites are comparable. Table 3.1 lists the information by letter for each population shown.

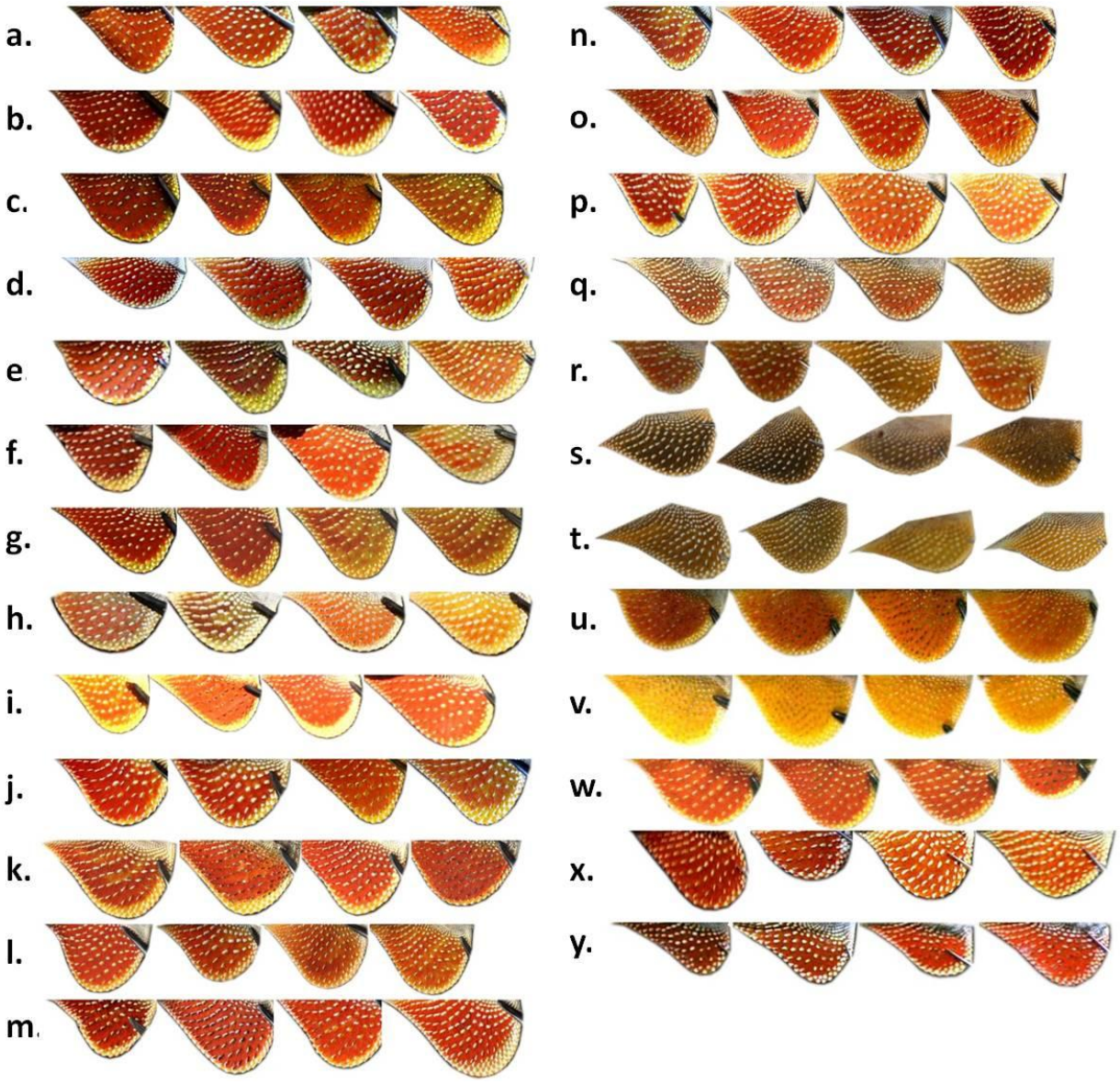

Figure 3.1 (b). Dewlap variation within populations. For each of the 25 populations we visited, four dewlaps are illustrated to demonstrate the variation among individuals within each population. Unlike in figure 1a, these images are not scaled so that dewlap size is comparable among populations.

Some sites were visited solely to collect data for this project, while data was collected incidental to other projects in some instances, so that not all variables were recorded at all sites. The variables collected at each site are listed in Table 3.1.

Dewlap measurements

The primary variables of interest in this study were the size, color, and pattern of the male dewlap. We measured the size and pattern of the dewlap from digital photographs of a minimum of ten males at each site extending the dewlap fully with forceps against a standard 17% grey background (Canon Rebel Xti, WhiBal®, PictureFlow LLC). We estimated dewlap size for each male from his photograph by measuring the area of the extended dewlap along the edge of the dewlap on one side and along a straight line on the other side using ImageJ v1.44 (Abramoff et al., 2004). We then regressed dewlap size against male body size (SVL) for the combined males from all sites to come up with an estimate of residual dewlap size for each male.

Photographs were also used to estimate dewlap pattern: the percentage of the dewlap that was red rather than yellow. To accomplish this, we measured the red portion of the dewlap three times and used the average, divided by the total dewlap area, to estimate the percentage of red in each dewlap. Prior to analysis, we arcsin-square root transformed the percent red estimate to conform to the expectation of normalcy (Sokal and Rohlf, 1995).

We also measured the color of the dewlap by recording reflectance at six sites on the dewlap; three sites in the center of the dewlap where the color tended to appear red, and three along the edge where the color appeared yellow, using an Ocean optics USB2000 spectrophotometer with a pulsed-xenon light source (Andersson and Prager, 2006). We characterized color from spectral data in several stages. First, we smoothed and condensed reflectance curves by averaging reflectance at 5 nm intervals across a length of the spectrum that exceeded the expected range of visual sensitivity for anoles (300 nm-700 nm) using the program CLR v1.05 (Montgomerie, 2008). Next, we calculated the average reflectance for each individual for the dewlap center and the dewlap edge. We then used visual modeling to condense each of these spectra into five

variables that may be meaningful to anoles. These variables were the estimated relative stimulation of each of the four types of cones found in the *Anolis* retina, plus the brightness of the dewlap relative to a white standard, estimated as the sum of the stimulation of the four photoreceptors. The stimulation value for each photoreceptor is a function of the sensitivity function of the photoreceptor, adjusted to account for absorbance by the oil droplet that filters light reaching the photoreceptor (Fleishman and Persons, 2001; Fleishman et al., 2009). We used the mean peak photoreceptor sensitivity, averaged from 17 species of *Anolis* (Loew et al. 2002), fit to Lamb's visual pigment template (Mansfield, 1985; Lamb, 1995) and adjusted to have no sensitivity below the 50% cutoff wavelength of the oil droplet associated with that photoreceptor type (also from Loew et al., 2002). More details about this approach can be found in Chapter 1.

The five color variables for each of two locations on the dewlap produced a total of ten highly correlated color variables. We further condensed these color variables using a principal components analysis (PCA). This allowed us to focus on the major axes of variation in color while reducing intercorrelation among variables. The average residual dewlap size, color and pattern data was calculated for all males from each site.

Sexual selection and sexual dimorphism

Secondary sexual traits like the dewlap are often thought to evolve in response to changes in sexual selection, although other factors are important (see Discussion). We used two variables that are thought to reflect differences in the strength of sexual selection at each site: sexual size dimorphism (SSD) and sexual dichromatism. Sexual selection is thought to favor male-biased SSD when large male body size is favored by intrasexual competition for access to mates (among other reasons: see discussion). To estimate SSD for each population, we collected at least ten adult animals of each sex and measured the snout-vent length of each using digital

calipers. We used the ratio between the average male size and the average female size as our estimate of SSD (VanHooydonck et al., 2009). Some authors have advocated alternative methods for reptiles because the distribution of adult body sizes in species with indeterminate growth can be non-normal (Stamps, 1993; 1995; Stamps et al., 1994; Butler et al., 2000), however our sample sizes at some sites were too limited to allow this approach. SSD was correlated with body size among populations, so we used the residuals of a regression of SSD on body size to make sure we were considering dimorphism only, rather than differences among populations in absolute body size.

Sexual dichromatism (SDC), the average difference between males and females in ornament color, is thought to be more exaggerated in species or populations under strong sexual selection since conspicuous male ornaments often evolve in response to mate choice or male-male competition (Barraclough et al., 1995; Badaeyev and Hill, 2003; Stuart-Fox and Owens, 2003; Wagner et al., 2012). To estimate SDC, we calculated the Euclidean distance between the male and female average at each site using the three PCA-generated color variables derived from spectral reflectance data.

Predation

Predation can increase the cost of visual signaling because visual predators can intercept signals intended for congeners (Zuk and Kolluru, 1998; Steinberg et al., 2014). We estimated predation intensity at each site by placing 100-400 model lizards on realistic perches and leaving them for three days before collecting them and looking for evidence of attacks. Models were cast of brown-pigmented beeswax in a mold made from a specimen of *A. carolinensis* (Figure 3.2a). We found clear evidence of attacks by birds, rodents, and other lizards, as well as ambiguous marks (Figure 3.2b-d). Since birds are the predators most likely to be sensitive to color variation,

only bird attacks were used to calculate predation rate (Brodie, 1993; Vignieri et al., 2010). Predation rate was calculated as the number of attacks per model per hour. The predation rate was arcsin-square root transformed prior to analysis (Sokal and Rohlf, 1995).

Species recognition

Experimental and comparative studies suggest that more complex dewlaps (those with a higher information content) may be favored in more diverse *Anolis* communities (Williams and Rand, 1977; Losos, 1985; Nicholson et al., 2007). We determined the presence and identity of congeners at each site by observation and by consultation with published range maps. We used the total number of congeners at each site as an input into our analyses.

Sensory drive and climatic effects

Differences in the quality of light and distribution of background colors in an environment can determine which signal colors are cryptic and which are more conspicuous (Endler, 1990). The climate at a site may determine what sort of vegetation will occur at that site and in turn, what the visual environment will be (Endler, 1990; 1993; Ng et al., 2013). Differences in climate among sites may also affect the spatial and temporal distribution of reproduction in a species, with consequences for the intensity or direction of social selection (Fitch and Hillis, 1984). For one or both of these reasons, climatic differences might be expected to influence ornament evolution. We estimated average climate at each site by extracting 19 bioclim variables at the 1km resolution from the worldclim dataset (Hijmans et al., 2005) using the Hawth's tools v3.27 with ArcMap v9.3 (Beyer et al., 2004; ESRI, 2008). We then used a PCA to capture the major axes of climatic variation among sampled populations.

Figure 3.2 (a) A wax model of *A. sagrei* used to estimate predation at different sites. This model is shown in situ at the bottom of the branch, below an actual adult male *A. sagrei*, showing the similarity in size, shape, and color between the models and live lizards. **(b)** Beak marks on a model suggesting an avian attack. **(c)** Bite marks from a rodent on a model. Such marks were not recorded as a predation attempt, both because predation on anoles by rodents is not thought to be common and because rodents are not primarily visual predators. **(d)** Bite marks on a model left in the field in Jamaica, apparently from a larger species of *Anolis*.

Invasion age

Older invasions have experienced selection in a novel environment longer than more recent invasions (and the native fauna has had longer to respond to *A. sagrei*), so we considered invasion age as a possible predictor of dewlap color. We determined the age of the introduction of *A. sagrei* at each site from multiple sources. Many of the populations we visited were the subjects of previous studies (Kolbe et al., 2004; 2007), and we used dates from those studies here. We also collected data from seven sites on small spoil islands where *A. sagrei* were deliberately released in 1995 as part of a separate study (Campbell, 1995). In some cases, such as in western Jamaica, it is unclear when *A. sagrei* became established and whether colonization was natural or human-mediated. We excluded populations that may not have been human-mediated from further analysis, although we illustrate the dewlaps from these populations in Figures 3.1, 3.2, and 3.4.

Do populations vary in dewlap morphology?

To determine if there is significant variation among populations in dewlap morphology (size, color and pattern), we conducted an Analysis of Variance in SPSS (v. 13.0, SPSS Inc, Chicago, IL, USA) for each dewlap variable using population of origin as our categorical variable. We conducted an ANOVA with all 25 populations, as well as just the 19 populations that have been recently introduced.

Genetic distance among populations

Populations that share a more recent common ancestor may be more similar than populations with more distant shared ancestry (Felsenstein, 1985; Harvey and Pagel, 1991). Typically, data that are phylogenetically structured can be analyzed using a variety of tree-based comparative methods, however, we are not able to use this approach because introduced populations of *A.*

sagrei are often the product of admixture between multiple introductions from diverse source populations (Kolbe et al., 2004; 2007) forming a complex reticulate phylogeny that is better represented as a network than a tree. Instead, we used haplotype data from a previous study (Kolbe et al., 2004, 2007) to calculate an average genetic distance between 14 populations using MEGA 5.1 (Tamura et al., 2011). We used the phytools package in R to conduct a matrix regression (Revell, 2011) to determine if genetic similarity could explain dewlap similarity. Dewlap similarity was determined as a distance matrix for each dewlap variable (residual dewlap size, pattern, and color PC1, PC2, and PC3) and a matrix regression was performed for each dewlap variable separately.

Explaining variation in dewlap size, color, and pattern among populations

We used the predictor variables suggested by our hypotheses (SSD, SDC, attack rate, climate PC1 and PC2, number of congeners), and invasion age as predictors of each of our five dewlap morphology variables. To determine which variables were the best at explaining dewlap variation we used a model selection approach. Specifically, we used SPSS to calculate the AICc score for all subsets of predictor variables and accepted the model with the lowest score (although we report the three best models for each variable).

Results

Dewlap color

The bulk of variation in dewlap color was explained by three principal component axes (Table 3.2). Color PC1, which accounted for 63.2% of the variation in the ten color variables, was high in dewlaps that were brighter but contained less red (Figure 3.3a). Color PC2 captured the contrast between the center and the edge of the dewlap: it was high in animals with bright

dewlap edges and dark centers (Figure 3.3b). Color PC3 was correlated with dewlaps with much red and UV coloration at the dewlap edge and little green and blue reflectance (Figure 3.3c).

Table 3.2. PCA statistics and loadings for dewlap color and climate among sites in this study.

	Color	**PC1**	**PC2**	**PC3**
	Eigenvalue	6.32	1.65	1.27
	% variance	63.18	16.47	12.66
Loading	Center brightness	0.51	0.16	0.78
	Center UVWS	0.85	-0.24	0.41
	Center SWS (blue)	0.95	-0.29	0.00
	Center MWS (green)	0.89	-0.38	-0.09
	Center LWS (red)	-0.94	0.32	-0.12
	Edge brightness	0.24	0.92	0.09
	Edge UVWS	0.63	0.50	0.19
	Edge SWS (blue)	0.92	0.05	-0.33
	Edge MWS (green)	0.80	0.22	-0.51
	Edge LWS (red)	-0.92	-0.30	0.23

	Climate	**PC1**	**PC2**
	Eigenvalue	10.16	5.08
	% variance	54.46	26.74
Loading	Bioclim 1 (mean annual temp.)	0.85	-0.51
	Bioclim 2 (mean diurnal range)	-0.78	0.33
	Bioclim 3 (isothermality)	0.96	-0.15
	Bioclim 4 (temp. seasonality)	-0.96	0.22
	Bioclim 5 (max. temp. warmest month)	-0.70	-0.32

Table 3.2 continued

Bioclim 6 (min. temp. coldest month)	0.93	-0.36
Bioclim 7 (temp. annual range)	-0.95	0.26
Bioclim 8 (mean temp. wettest quarter)	-0.41	-0.66
Bioclim 9 (mean temp. driest quarter)	0.95	-0.23
Bioclim 10 (mean temp. warmest quarter)	-0.25	-0.78
Bioclim 11 (mean temp. coldest quarter)	0.92	-0.36
Bioclim 12 (annual precip.)	0.83	0.52
Bioclim 13 (precip. wettest month)	0.87	0.20
Bioclim 14 (precip. driest month)	0.26	0.95
Bioclim 15 (precip. seasonality)	0.32	-0.79
Bioclim 16 (precip. wettest quarter)	0.83	0.30
Bioclim 17 (precip. driest quarter)	0.32	0.91
Bioclim 18 (precip. warmest quarter)	0.14	0.21
Bioclim 19 (precip. driest quarter)	0.60	0.56

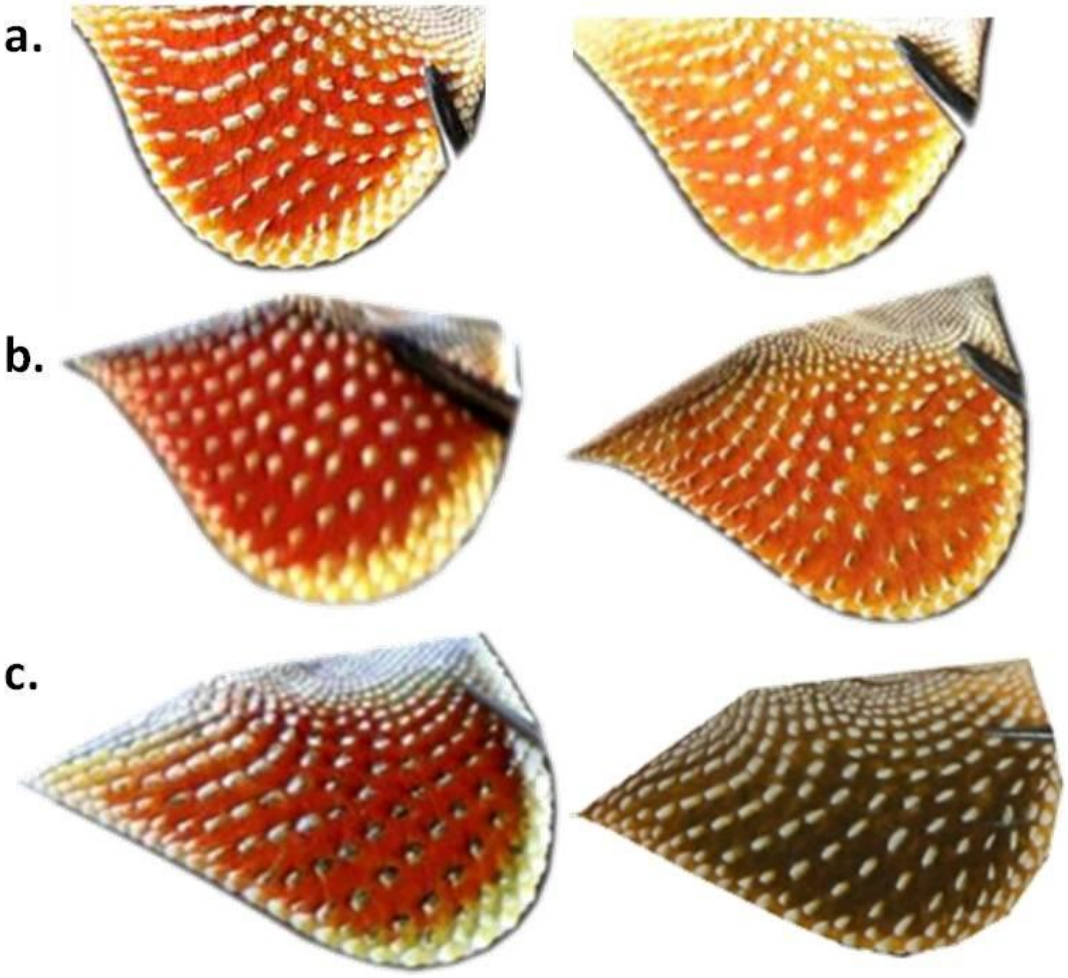

Figure 3.3. An illustration of dewlaps with high and low values of color PC scores. Loadings for each are shown in Table 2. **(a)** The difference between a dewlap with a low value of color PC1 (left) and a high value (right). Color PC1 loaded heavily on the amount of red in the center and the edge of the dewlap. **(b)** The difference between a dewlap with a low value of color PC2 (left) and a high value (right). Low values correspond to greater contrast between the color of the center of the dewlap and the edge while contrast is reduced in dewlaps with high scores. **(c)** The difference between a dewlap with a low value of color PC3 (left) and a high value (right). High values correspond to more UV reflectance in the dewlap center.

Variation among populations in dewlap morphology

There was significant variation among populations in every dewlap variable we measured (Table 3.3; Figure 3.4), whether we considered all populations sampled, or just recently introduced populations. We found enormous variation in relative dewlap size, color, and pattern among recently introduced populations of *A. sagrei* (Table 3.3). In recently introduced populations, the largest and smallest dewlaps were both found in populations in Florida (e: largest, h: smallest), while other populations spanned the entire range of variability (Figure 3.1a & b, 3.4a). The percentage of the dewlap covered with red (pattern) ranged from 70% (population m) to 87% (population i), though in some populations it was difficult to determine how to delineate the center coloration from the edge, either because the dewlap was more solid in color (e.g. populations d & x) or because stripes of yellow interrupted the red region (e.g. populations c & g) (Figure 3.1a & b, 3.4b). Color PC1, 2, & 3 were also highly variable among populations, with less red in both the center and the edge of the dewlap (color PC1) in populations such as e and f, and more red in populations such as x and i (Figure 3.1a & b, 3.4c). Color PC2, which reflected greater contrast between the center and the edge of the dewlap, was especially pronounced in populations d, e, g, and k, and more muted in f and w (Figure 3.1a & b, 3.4d). The brightness and amount of UV in the center of the dewlap, color PC3, was high in populations e and h, and low in w and y (Figure 3.4e).

Table 3.3. ANOVA results by variable for all populations, and recently introduced populations only: dewlap variation among populations exceeds variation within populations for all dewlap variables considered.

Dewlap variable	F (all populations)	p	F (recent only)	p
Residual dewlap size	15.91	<0.0001	13.83	<0.0001
Pattern	6.2	<0.0001	8.18	<0.0001
Color PC1	32.63	<0.0001	24.04	<0.0001
Color PC2	64.92	<0.0001	28.71	<0.0001
Color PC3	34.73	<0.0001	22.89	<0.0001

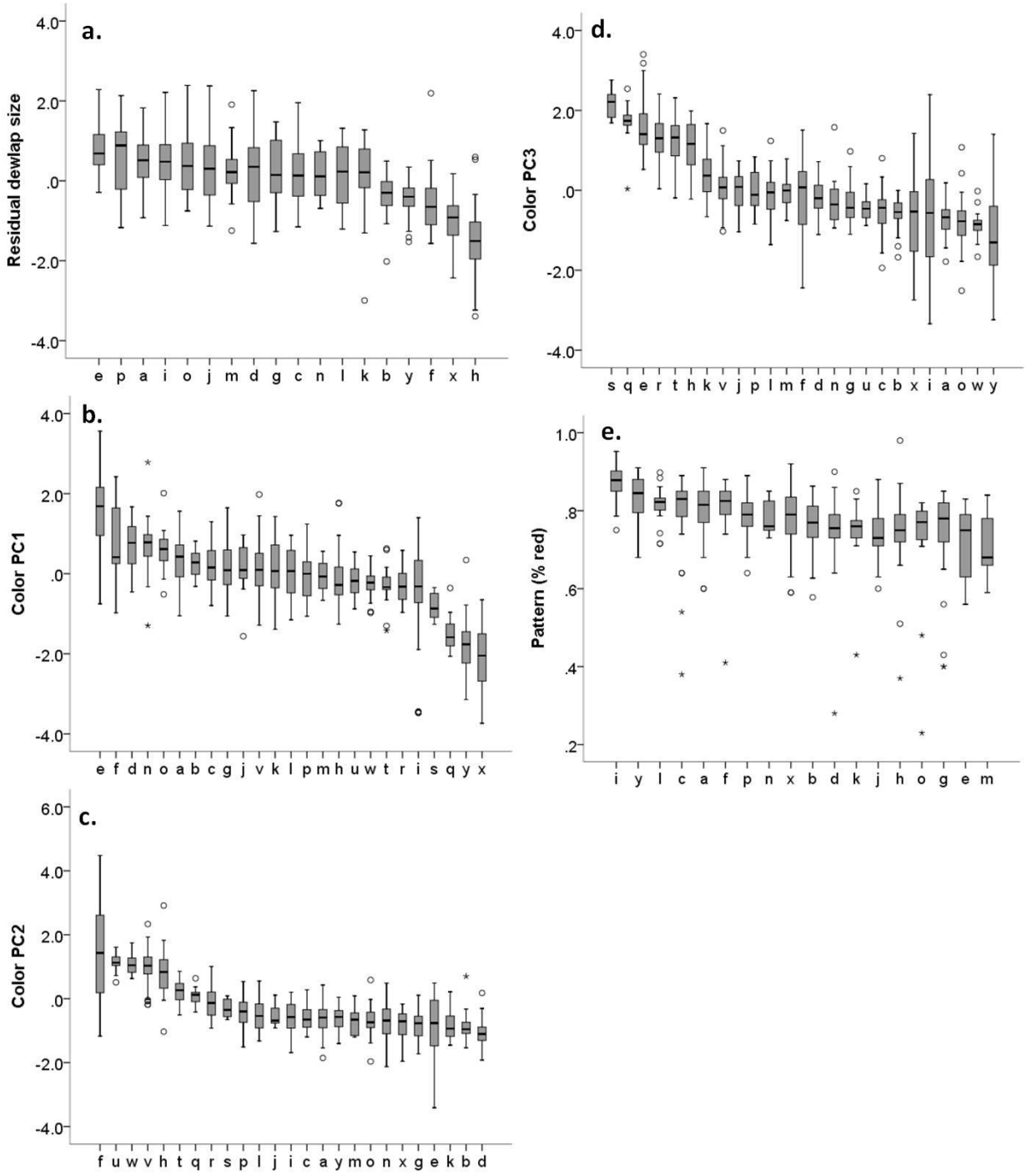

Figure 3.4. Variation among populations in the mean and variance of dewlap size **(a)**, dewlap color PC1 **(b)**, dewlap color PC2 **(c)**, dewlap color PC3 **(d)**, and dewlap pattern **(e)**. Letters correspond to Figure 3.1 and Table 3.1.

It is worth noting that we observed more extreme values of all dewlap variables in populations that were not the consequence of recent introductions. The smallest relative dewlap sizes measured were found in Honduras (q, r, s, and t) and Jamaica (x), and the smallest percentage of center color were found in Honduras (q and r) and on Cayman Brac (v). The lowest values of color PC1 were observed in Jamaica and Honduras (q, x), while for color PC3 the highest (q, s) and lowest (x, w) values were measured in populations that were not recently introduced. These results may suggest that older populations have experienced more evolutionary change than younger populations, although we cannot rule out alternatives such as that the source of these populations influenced their trajectory.

Genetic distance and dewlap dissimilarity

Among populations, genetic similarity was correlated with relative dewlap size and color PC1 (Table 3.4). PC2 and PC3 were not significantly correlated with genetic relatedness among populations. Due to the unique history of the populations we studied and the drawbacks of the method used to estimate phylogenetic signal, we may have underestimated these relationships (see discussion).

Correlates of Dewlap variation:

Dewlap size: 80% of the variation in dewlap size was explained by two variables in the best model, SDC and invasion age (Table 3.5). There was a negative relationship between residual dewlap size and SDC, such that populations in which the male dewlap was smaller were also more dichromatic (Table 3.6, Figure 3.4). Older populations also have smaller relative dewlaps.

Dewlap pattern: 18% of the variation in dewlap pattern was explained by just one variable, predation rate (Table 3.5). In other words, sites with higher predation also had a larger percentage of the dewlap surface covered with red, rather than yellow (Table 3.6).

Color PC1: The best model to explain color PC1 contained four variables: SSD, predation, climate PC2, and number of congeners. This model explained 87% of the variation in color PC1 (Table 3.5). Sites with higher SSD had more red in the dewlap (Table 3.6, Figure 3.5a). Sites with fewer congeners also had more red (Figure 3.5b). In contrast, sites with higher predation and less seasonal climates had less red (Figure 3.5c,d).

Color PC2: None of the models for color PC2 explained significant variation in this aspect of dewlap color (Table 3.5).

Color PC3: invasion age was the best predictor of color PC3, explaining 46% of the variation in this trait (Table 3.5, Figure 3.6), although this predictor was only marginally better than the alternative models.

Table 3.4. Genetic distance and dewlap dissimilarity for five aspects of dewlap morphology.

Dewlap variable	**r^2**	**Coefficient for dewlap variable**	**t (dewlap variable)**	**P (dewlap variable)**
Residual dewlap size	0.13	5.12	3.36	0.01
Pattern	0.01	-0.11	-0.88	0.60
Color PC1	0.15	7.15	3.63	0.04
Color PC2	0.02	2.99	1.39	0.10
Color PC3	002	1.81	1.34	0.52

Table 3.5. Top three models to predict variation in dewlap size, pattern, and color.

Variable	Model rank	Model terms	AICc	r^2
Residual dewlap size	1	SDC, invasion age	-33.42	0.80
	2	SDC	-32.74	
	3	SDC, congeners	-32.18	
Pattern	1	Predation	-82.32	0.18
	2	Invasion age	-82.10	
	3	None	-81.40	
Color PC1	1	SSD, predation, climate PC2, congeners	-25.92	0.87
	2	Climate PC2, congeners	-24.78	
	3	Predation, climate PC2, congeners	-23.18	
Color PC2	1	None	-44.64	0.00
	2	Climate PC2	-43.89	
	3	Climate PC3	-43.18	
Color PC3	1	Invasion age	-27.69	0.46
	2	Invasion age, congeners	-27.66	
	3	Invasion age, climate PC1	-27.44	

Table 3.6. Coefficients for the top model for each dewlap variable.

Dewlap variable	Model term	coefficient
Residual dewlap size	SDC	-6.98
	Invasion age	0.02
Pattern	Predation	0.21
Color PC1	SSD	-0.06
	Predation	2.33
	Climate PC2	0.38
	congeners	-0.64
Color PC2	None	n/a
Color PC3	Invasion age	0.03

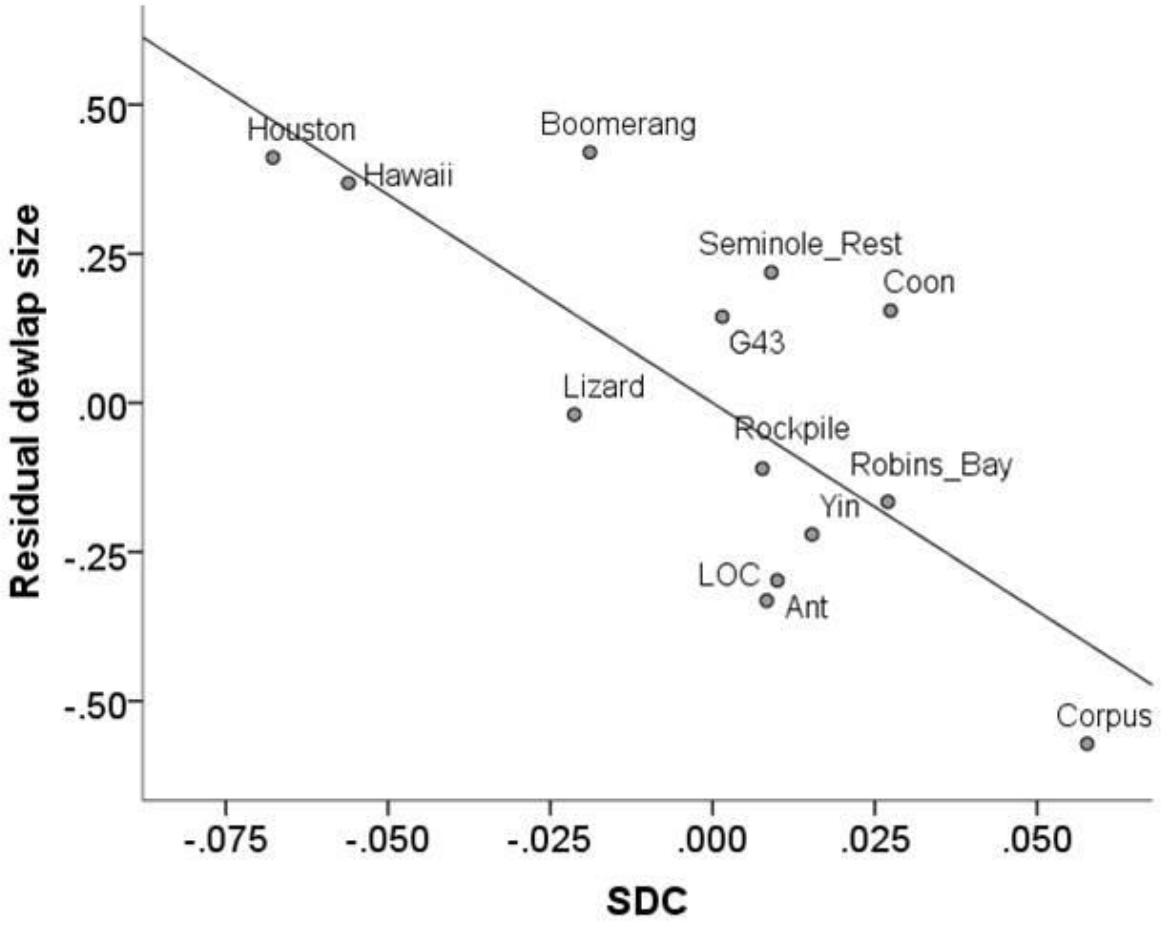

Figure 3.5. The relationship between relative dewlap size and sexual dichromatism (SDC) for 13 recently introduced populations of *A. sagrei*. SDC alone explained 80% of the variation among populations in relative dewlap size. Site names are listed in table 3.1.

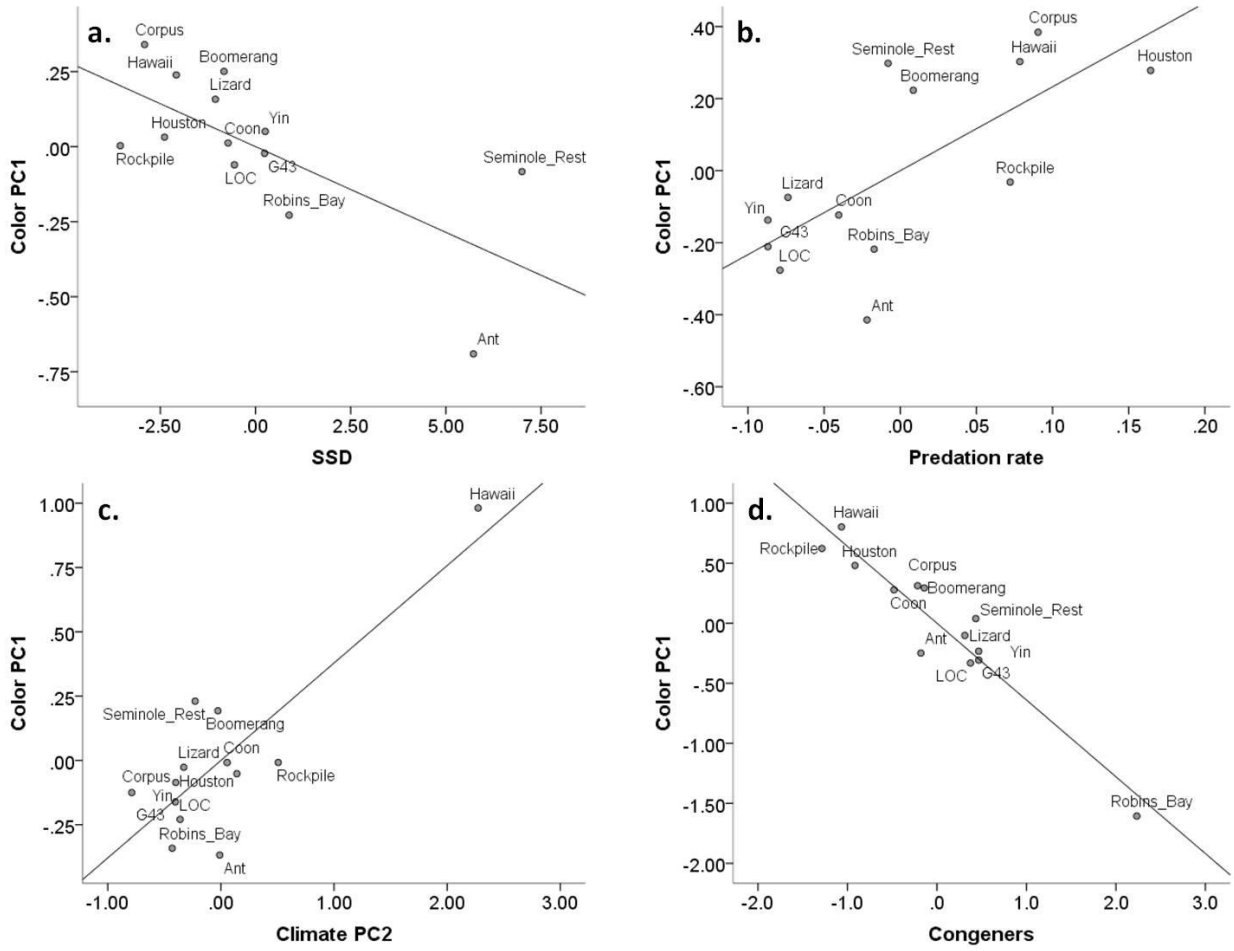

Figure 3.6. Partial regression plots for four predictor variables identified in the best model to explain variation in color PC1: sexual size dimorphism (SSD) **(a)**, predation **(b)**, climate PC2 **(c)**, and congeners **(d)**. Overall, the model explained 87% of the variation among populations in color PC1.

Discussion

Colorful ornaments have attracted enormous interest from behavioral and evolutionary ecologists because of the critical role they play in interactions within and among species, and their suspected role in species diversification (e.g. Barraclough et al., 1995; Ritchie, 2007; Wagner et al., 2012). Evolutionary change of animal ornaments is thought to result from a variety of processes, such as sexual selection, predation, species recognition, and sensory drive (West-Eberhard, 1983; Endler, 1984; Andersson, 1994; Bradbury and Vehrencamp, 1998). Introduced species offer a powerful opportunity to understand processes of ornament diversification because they can reveal the earliest stages of evolution under conditions that favor rapid change (Huey et al., 2005; Velland et al., 2007).

We investigated variation in the size, color, and pattern of the dewlap among recently introduced populations of *A. sagrei*, and explored whether this variation can be explained by various predictors suggested by the literature. We found unexpectedly large variation among populations, far beyond variation within populations (Figure 3.1, 3.4), or in other *Anolis* species (Losos, 2009). There was significant among-population variation in dewlap size, color, and pattern whether we considered only recently introduced populations or included older populations of uncertain origin.

Previous studies in *Anolis* have shown that variation in color and pattern of the dewlap is heritable, and does not change in response to dietary restriction (Steffen et al., 2010; Ng and Glor, 2011). Although these studies were not conducted in *A. sagrei*, they suggest that dewlap differences among individuals and populations are more likely to result from genetic differences than environmentally-mediated phenotypic plasticity. [what is the size of the heritability? If

heritability is measured within populations this does not tell you whether variation among populations is heritable.]

The dewlap variation we find is almost unprecedented in anoles (Losos, 2009). One exception is the Hispaniolan trunk anole, *A. distichus*, which shows substantial variation in dewlap color both within and among populations (Case and Williams, 1984; Williams and Case, 1986). In the case of *A. distichus*, some phenotypically distinct populations are genetically divergent while others are not (Case and Williams, 1984; Williams and Case, 1986; Ng and Glor, 2011). Dewlap color and gene flow among adjacent populations both seem to depend on climatic variation among sites (Ng et al., 2013). However the *A. distichus* complex is ancient, and geographically distinct populations have had a long time to diverge (Case, 1990). In this study, the populations we considered were established very recently (<150 years) through human-mediated dispersal. Variation among these populations likely represents either very rapid divergence, or pre-existing variation from within the natural range among the founders of different populations. We consider each of these possibilities below.

Potential explanations for intraspecific dewlap variation

We are able to explain a substantial amount of the intraspecific dewlap variation in *A. sagrei* using a set of predictor variables chosen based on four hypotheses to explain interspecific variation in dewlap morphology among anoles. We review the relationship between dewlap morphology and each group of predictor variables in turn.

Sexual dimorphism

We found that dewlaps were smaller in populations with high sexual dichromatism (SDC: Figure 3.5), and that dewlaps were redder (color PC1) in populations with greater sexual

dimorphism in size (SSD: Figure 3.6a). These patterns suggest several possible mechanisms of dewlap divergence deserving further research.

Large, colorful ornaments and sexual dimorphism in size or color are often attributed to sexual selection, leading to the expectation that ornament characteristics may be correlated with dimorphism among populations or among species. SSD or SDC could evolve by sexual selection if dewlap size and color affect the outcome of either male-male competition or female choice. Populations may diverge because sexual selection is stronger or differs between environments. For example, when resources are distributed in clumps, males can monopolize several females by defending territories that encompass resources, while females may have more opportunities to exercise mate choice in resource-rich environments where male densities are high (Emlen and Oring, 1977). Sexual selection could also produce divergence among populations if female preferences vary among populations depending on the environment (e.g. sensory drive), or if females prefer novelty and different novelties arise by chance in each population.

It is important to note that SSD can evolve in response to factors other than sexual selection. Greater SSD may evolve to reduce competition for resources between the sexes (Stamps et al., 1997; Butler et al., 2000; Butler and Losos, 2002), and female-biased SSD can evolve as a consequence of fecundity selection (Stamps et al., 1997; Cox et al., 2003). These factors complicate the interpretation of our findings.

It is possible that the correlations we observe between sexual dimorphism and dewlap size and color are a consequence of sexual selection. For example, SSD and red coloration in the dewlap may both be favored by strong sexual selection if larger, redder males are better at winning contests against other males or attracting females. Alternately, if SSD evolved to reduce intersexual resource competition and this correlates with increased intrasexual resource

competition, the correlation between SSD and dewlap color could reflect a role for the dewlap in mediating male-male competition for resources other than mates.

The relationship between SDC and dewlap size is more difficult to interpret. Strong sexual selection seems likely to produce larger male signals and greater color differences between males and females, while we find the opposite correlation. Again, the difficulty here results from using SDC as an estimate of the strength of sexual selection. SDC can be exaggerated in a population either because males become more conspicuous or females become less conspicuous (Amundsen, 2000), suggesting that selection on female coloration could also influence this relationship. For example, a high rate of predation could favor duller coloration in females and smaller (but equally colorful) ornaments in males. In this study, SDC was more strongly correlated with male color than female color, suggesting that selection on females is not responsible for the observed pattern (Supplementary Table S1). Alternatively, populations experiencing strong sexual selection evolve either larger or more colorful male ornaments, but genetic or ecological constraints (e.g. predation) make it difficult to achieve both.

Using morphological proxies to capture the strength of sexual selection is tricky, but the strong correlations we find between sexual dimorphism and dewlap morphology suggest that it would be worthwhile to explore the interplay between dewlap evolution and sexual selection in more detail, perhaps by measuring sexual selection on the dewlap directly in various populations. Although this approach will require a substantial effort, our results suggest that such effort could result in valuable insights into *Anolis* dewlap evolution.

Avian predation

Conspicuous displays may attract the attention of predators. We estimated the intensity of avian predation by recording the rate of bird attacks on wax models (Figure 3.2; Brodie, 1993;

Vignieri et al., 2010). Our data suggest that populations of *A. sagrei* that experience higher avian predation have less red and yellow in their dewlaps (Figure 3.6b). It is plausible that this reduces the risk of predation to these males by making their dewlaps less conspicuous to birds, though it would be necessary to model how avian visual systems will respond to dewlap differences under the prevailing visual conditions among sites to confirm this. Previous studies have shown that anoles attempt to evade the notice of predators by adjusting the amplitude of their displays and the time spent displaying when predators are present (Cantwell and Forrest, 2013; Driessens et al., 2013; Steinberg et al., 2014). While our results could be a consequence of further attempts to evade predation, dewlap color could also change to compensate for a muted motion component of the display, i.e. males can make up for moving less by having more conspicuous coloration. It would be interesting to investigate display behavior in the populations considered here to further clarify how anoles balance the risk of predation with the necessity of intraspecific communication.

Anoles may not always try to avoid the notice of predators: some species display their dewlaps to deter pursuit by predators (Leal and Rodruiguez-Robles, 1995; 1997; Leal, 1999). Our results could also be explained if particular colors or patterns are more effective at deterring predators, although it is not clear why this should be the case. Red and yellow are often found in aposematic signals, but anoles are so ubiquitous and so visually different from anything else that dewlaps seem unlikely to have any value in mimicry of a dangerous animal; moreover, anoles are frequently eaten by predators of all stripes (Losos, 2009). Vanhooydonck et al. (2009) concluded that pursuit-deterrence could explain larger dewlaps in Bahamian populations of *A. sagrei* in sympatry with the saurian predator, *Leiocephalus carinatus*. Presumably larger dewlaps deter predators more effectively, either because they are better at transmitting the pursuit-

deterrence signal to predators, or because a large dewlap suggests that an individual anole is unlikely to make good prey because it is especially fast or strong (see Henningsen and Irschick (2012) for evidence that dewlap size is an honest indicator of bite force). We did not find a relationship between estimated predation and dewlap size in our data, perhaps because pursuit-deterrence is less effective as a signal to avian predators than to other types of predators. It would be instructive to combine behavioral observations of predators with visual modeling of how conspicuous different dewlaps would appear to a variety of predators to determine what signals would function best for pursuit-deterrence with different communities of predators.

The Anolis community

Dewlaps are widely believed to function as cues of species identity in complex anole communities. Our data support the idea that color PC1 is related the complexity of anole communities (Figure 3.6d). Sites with more species had more red in the dewlap, which could increase the distinctiveness of the *A. sagrei* relative to sympatric anoles (depending on the dewlap color of sympatric species). In most populations we visited, the dewlap of *A. sagrei* was already very distinct from other *Anolis* species present, so it is not clear that selection would necessarily lead to even larger differences among species. For example, the most common species at our Jamaican sites were *A. grahami*, *A. lineatopus*, and *A. opalinus* which have yellow-orange, white-orange, and yellow-orange dewlaps, respectively. Although the deep red dewlap of *A. sagrei* in the two populations in Jamaica would probably make them more distinct, it seems unlikely that this would be necessary since no native Jamaican anoles have red and yellow dewlaps. On the other hand, the *A. sagrei* dewlap has become far less red in several populations where it does not co-occur with any sympatric anoles, such as the Swan Islands and Cayman Brac. Although these populations were not included in our analysis because of their

probable ancient origin, they suggest that a trend towards less red in the dewlap may be more widely observed in *A. sagrei* when they occur in isolation. This pattern was suggested by Rand and Williams (1970), who observed that monospecific anole communities in the Lesser Antilles often converge on a simple yellow-orange dewlap.

Another possibility is that sympatric anoles exert selective pressure on *A. sagrei* through a mechanism other than species recognition. For example, larger anoles can be predators of smaller species. In Jamaica, the most diverse *Anolis* community we sampled in this study, *A. garmani* consumes adult *A. lineatopus* (which is larger on average than *A. sagrei*; Gerber, 1999). In fact, we found clear evidence of anole attacks on our wax models when measuring predation at both sites in Jamaica (Figure 3.2d). Differentiating between species recognition and anole-on-anole predation will require further investigation. One possible approach would be to conduct staged encounters between sympatric species (as in Losos, 1985) to determine if small shifts in dewlap color can increase or decrease antagonistic interactions between pairs of species.

Whether or not selection for species recognition is currently shaping divergence of the dewlap among populations of *A. sagrei*, an exciting possibility is that changes in the dewlap might play a role in future reproductive isolation among populations that have diverged in dewlap morphology (exciting, at least, to those of us who think the world could use more anole species). Comparative, observational, and experimental evidence all suggest that dewlap color plays a role in species recognition in anoles (Rand and Williams, 1970; Losos, 1985; Webster and Burns, 1973; Nicholson et al., 2007 Lambert et al., 2013). However, differences in dewlap color do not always correlate with reproductive isolation within species. (Ng et al., 2013; Stapley et al., 2011). Since no studies have investigated how *A. sagrei* recognize conspecifics, it remains

to be seen if dewlap variation among populations could someday lead to reproductive isolation between divergent populations.

Climatic effects

In this study, color PC1 and climate PC2 are correlated. Dewlaps are redder in sites with more seasonal precipitation . The sensory drive hypothesis suggests that the light environment may determine what color is most efficacious for signals, with vegetation the primary determinant of light environment on land (Endler, 1990; 1993). At a large scale, climate may determine the vegetation structure at a site, and thus may predict the availability of different light environments at that site (Ng et al., 2012). For example, Leal and Fleishman (2004) found that populations of *A. cristatellus* in xeric environments experienced higher light and had darker dewlaps than populations in mesic environments. In the present study, it may be the case that seasonal vegetation is more open than vegetation in less seasonal environments and that this favors more red in the dewlap of *A. sagrei*. However, the relationship between climate and habitat light has not been conclusively demonstrated. Furthermore, at many sites where introduced *A. sagrei* thrive, the landscape has been heavily modified and maintained by people and may therefore not reflect local climatic conditions (personal observation). In fact, at a subset of the sites considered in this study, we recorded the percent canopy cover of perches where male *A. sagrei* were observed, as well as the perch height and diameter employed by these males (Supplementary materials). If climate is a strong predictor of vegetation structure, we should expect correlations between the climate variables we include here and canopy cover and perch characteristics. We did not find correlations between any of these variables (Supplementary Table 3.2). The conclusion that sensory drive can explain the relationship between climate and dewlap color should be considered tentative at best.

An intriguing alternative to explain how climate might affect ornament evolution, first proposed by Fitch and Hillis (1984), is that seasonal environments limit the duration of the mating season and thereby increase the intensity of sexual selection. This possibility could be explored with interspecific comparative studies and more detailed characterization of sexual selection within populations.

Genetic distance

For the subset of populations where genetic data were available, we found that genetic similarity was correlated with variation in dewlap size and color PC1, a pattern known as phylogenetic signal (Table 3.4). Traits evolving according to a Brownian motion process (like genetic drift) will have high phylogenetic signal, while the absence of phylogenetic signal can imply adaptive evolution (e.g. traits evolving by an OU process) or rapid evolution within bounded trait space (Gittleman et al., 1996; Blomberg et al., 2002; 2003; but see Revell et al., 2008 for complications with interpretation). The method we used to measure phylogenetic signal has lower power than the approach used by Blomberg et al. (2002; 2003), and could underestimate phylogenetic signal in our sample (Harmon and Glor, 2010). However, in this case no feasible alternative was available due to widespread admixture in invasive populations. Genetic admixture creates further problems with interpreting phylogenetic signal in two ways. First, the novel genetic combinations produced through admixture could produce unique phenotypes via epistatis. Second, the genetic distances we calculated between pairs of populations were based on the average distance among individuals from these populations, but these distances are unlikely to be normally distributed when populations overlap in some, but not all, of their source populations. Population admixture is more likely to obscure phylogenetic

signal in a trait rather than create the illusion of signal where there is none, so it is likely that there is more phylogenetic signal in dewlap morphology than we estimate here.

Because of the methodological and biological complications of estimating phylogenetic signal with our data, our conclusions about the effect of genetic relatedness on dewlap similarity are necessarily tentative. However, the fact that we find phylogenetic signal for dewlap size and color PC1 in spite of these limitations suggests that population history plays at least some role in determining dewlap variability among populations. In future studies, it would be interesting to look at the evidence for phylogenetic signal for dewlap variables within the native range of this species or others, where the population history is not obscured by complex patterns of human-mediated dispersal and where superior methods to estimate phylogenetic signal could be used.

Conclusion

The populations of *A. sagrei* considered in this study show unexpected variation in the size, color, and pattern of the dewlap, especially considering that many of these populations are the result of recent anthropogenic introduction. We were able to explain a large percentage of the total variation in dewlap size, color, and pattern using variables suggested by the literature, including sexual dimorphism, predation, community complexity, and climatic effects. In contrast, very little of the variation among populations is explained by the genetic relatedness of populations, although the complex history of admixture in source population complicates interpretation. The high proportion of variation explained by ecological variables, the apparent lack of phylogenetic signal, and the recent origin of our populations suggest that independent introductions of *A. sagrei* may be diverging rapidly as a consequence of adaptive evolution. Although it is difficult to infer causal relationship from the correlations between these variables

and dewlap morphology, the patterns we observe suggest several directions for future research efforts.

Chapter 4

Evolution of an ornament, the dewlap, in females of the lizard genus *Anolis*[1]

Abstract

Male ornaments have been the subject of numerous studies on sexual selection and communication, but female ornaments have garnered substantially less study, even though female ornaments are well developed in some species. The factors that have propelled the evolution of elaborate ornaments in females are poorly understood, but may include genetic byproducts, social selection, habitat differences, or species recognition. We used simulation-based comparative methods and a newly estimated phylogeny to test these four hypotheses to explain female ornamentation within the diverse neotropical lizard genus *Anolis*. We found support for the habitat difference hypothesis and the social selection hypothesis; the female dewlap was larger in species that use more arboreal habitats, and in species where the sexes were less dimorphic. We did not find support for the genetic byproduct hypothesis or the species recognition hypothesis. We propose that the size of the female dewlap may evolve in response to differences in habitat use, and social selection such as male mate choice or intrasexual competition for territory among females.

Key words: *Anolis*, signaling, ornament evolution, sensory drive, species recognition, comparative methods, social selection

[1] Reprinted from: Harrison, A., & Poe, S. (2012). Evolution of an ornament, the dewlap, in females of the lizard genus *Anolis*. *Biological Journal of the Linnean Society*, 106(1): 191-201.

Introduction

Animal ornaments are some of the most astonishing structures in the natural world. Male ornaments have been shown to function as badges of status or indicators of condition among competing males, and also as signals to attract females (Andersson, 1994; Alcock, 2001). Males also use ornaments to advertise species identity (Andersson, 1994; Alatalo et al, 1994), and to communicate alertness to predators (Caro, 1995). In many species, females also possess ornaments. In some cases, female ornaments may function exactly as male ornaments do, particularly in species that demonstrate sex-role reversal (reviewed in Eens and Pinxten, 2000). Yet in species where sex roles are not reversed, the function of female ornamentation remains unclear, in part because most studies of ornament evolution focus on males.

Four main hypotheses have been advanced to account for the occurrence of female ornaments. Early studies on ornamentation assumed that female ornaments were a byproduct of selection on males (the genetic byproduct hypothesis: Darwin, 1871; Lande, 1980; Amundsen, 2000a, 2000b). In other words, females may develop male ornamentation because selection on one sex will lead to a correlated response in the other sex (Lande, 1980, Amundsen, 2000b). However, comparative studies in birds and lizards have shown that transitions from sexual monochromatism to dichromatism are common (birds: Amundsen, 2000b; lizards: Wiens, 1999; Ord and Stuart-Fox, 2006), which indicates that male and females ornaments can evolve in tandem, but may also be decoupled when dimorphism is favored by selection.

A second hypothesis to explain female ornaments is social selection, which invokes either sexual selection acting on females or territorial defense of nest sites, territories, or other resources critical to reproduction, to explain the exaggeration of female ornaments (West-

Eberhard, 1983). A male preference for showy females has been demonstrated in the two-spotted goby, a fish(Amundsen and Fosgren, 2001) and some birds (Amundsen, 2000b). However in an equal number of bird species, males showed no preference or experimental results were equivocal (Amundsen, 2000b). Studies on the role of female ornaments in territorial defense are limited; in at least one species, the size of a female ornament was shown to affect the outcome of female-female competition for nest sites (West-Eberhard, 1983), but more studies of female territorial interactions are needed before the generality of this hypothesis can be assessed.

Aside from direct studies of behavior, the role of social selection in female ornament evolution might be studied by looking for correlations between the degree of elaboration of a female ornament and other traits that reflect the social or mating system of a species. One such trait may be sexual size dimorphism (SSD). Male-biased SSD (where males are larger than females) is most likely in polygamous mating systems where males compete for access to groups of females in contests of strength and endurance (Emlen and Oring, 1977; Andersson, 1994). This may evolve in response to the spatial and temporal distribution of females: when receptive females are concentrated groups can be monopolized and defended by a single dominant male. When females are spread widely through the habitat, large numbers cannot be monopolized by a single male and large size might be unnecessary or even detrimental to males that need energy to expend on searching for mates (Emlen and Oring, 1977). It follows that SSD will be correlated with the spatial distribution of females and potentially with other socially relevant factors such as female territoriality. This makes SSD a useful, if imprecise, proxy variable for characterizing the mating system of a species, especially for species where little is known about behavior and social interactions in the wild. This is a variable that should be used with caution, however, since SSD

may also reflect niche partitioning between the sexes, fecundity selection, or other processes (e.g. Butler et al., 2000).

Selection for species recognition has also been suggested as an explanation for variation in female ornamentation (Williams and Rand, 1977; Paterson, 1985; Amundsen and Pärn, 2006). Reliable species recognition mechanisms may be important to prevent interspecific mating, courtship, or aggression, which can be costly to both males and females. The ability to correctly identify conspecifics will be especially important in communities where closely related species co-occur, particularly if mating signals are similar among species, because the potential for mistakes is greater under these conditions. The species recognition hypothesis has not been explicitly tested for females, although male ornaments have been shown to function in species recognition in a wide variety of organisms (e.g. Losos, 1985; Searcy and Brenowitz, 1988; Alatalo, 1994; Hankison and Morris, 2002). Interspecific hybridization is generally believed to be more costly to the female, which may explain why species recognition cues are more common in males.

A fourth hypothesis to account for the evolution of female ornaments is that differences in habitat could exert differential selective pressures on ornaments. One way that this could happen is via sensory drive, which posits that optimal signal characteristics will vary depending on the environment (Endler, 1990; Endler, 1993). What constitutes a conspicuous signal under one set of environmental conditions may be cryptic in a different context. For example, in dim environments large, bright signals will be favored while in bright habitats smaller, dark signals will be more effective at attracting attention (e.g. Leal and Fleishman, 2002). Differences in the degree to which a given signal is cryptic or conspicuous in a given environment can be

exacerbated by adaptation of the receiver's sensory system to the new environment (Endler and Basolo, 1998).

However sensory drive is not the only way that habitat can affect ornament evolution - other agents of selection such as predators and prey may also differ between habitats and this may also lead to changes in ornaments. In a study of agamid lizards, Stuart-Fox and Ord (2004) found that the number of ornaments in both males and females was correlated with habitat openness. The authors of this study suggest that this correlation might be the result of habitat differences, or, alternately, differences in predation pressure among different habitats could produce this pattern. Habitat differences might therefore lead to ornament evolution via multiple routes.

Lizards in the neotropical genus *Anolis* provide an ideal opportunity to test these hypotheses in a phylogenetic comparative context. Nearly all of the approximately 400 species of anole use an erectable flap of colorful skin on the throat, the dewlap, for communication. The size and the color of both the male and female dewlap vary greatly among species, though in many species females lack a dewlap altogether (Williams and Rand, 1977; Nicholson et al., 2007). Moreover, anoles utilize a diverse array of habitats and social behaviors (Losos, 2009). The phylogenetic relationships among species are also relatively well known (Poe, 2004; Nicholson et al., 2005), although mainland species have been underrepresented in past studies.

The dewlap is extended by males while courting females and defending territories (e.g. Jenssen et al., 2000); they may also display the dewlap when they become aware of the approach of a predator (Leal and Rodriguez-Robles, 1997). Male dewlap color has also been suggested to facilitate species recognition (Williams and Rand, 1977; Losos, 1985). Habitat characteristics such as the light environment are also correlated with male dewlap color (Leal and Fleishman, 2002) and size (Losos and Chu, 1998). In summary, the evidence suggests that sexual selection,

species recognition, and sensory drive are all involved in the evolution of the dewlap of male *Anolis*.

Female dewlap size in anoles ranges from complete absence to very large (Figure 4.1; Fitch and Hillis, 1984). When present, the female dewlap usually exhibits the same coloration as the male, but in a few species the male and female dewlaps may differ dramatically in color or pattern (e.g. *A. fitchi*, *A. lyra*: Figure 4.1.; *A. chocorum, A. lemurinus*: Savage, 2002; *A. johnmeyeri*: Köhler, 2003; *A. nitens*, *A. transversalis*: Bartlett and Bartlett, 2003; *A. insignis*: Losos, 2009). For species where the female dewlap is well developed, female displays have been observed in the absence of other lizards (*A.* Chamaeleolis *barbatus*: Leal and Losos, 2000; *A. fowleri*: L. Mahler pers. comm.) or during unsuccessful courtship attempts by conspecific or heterospecific males (*A. valencienni*: Hicks and Trivers, 1983). Like males, females often defend exclusive territories which suggests that social selection may operate on female anoles, but the dewlap is not critical to female territoriality, as many species lacking a female dewlap defend territories using push-up and head-bob displays (e.g. *A. lineatopus*: Rand, 1967; *A. nebulosus*: Jenssen, 1970; *A. polylepis*: Andrews, 1971; *A. aeneus*: Stamps, 1973; *A. garmani*: Trivers 1975; *A. carolinensis*: Nunez et al., 1997: *A. bahorucoensis*: Orrell and Jenssen, 1998). In a recent survey of the diversity of male dewlap colors and patterns, Nicholson et al. (2007) suggest that female behavior or habitat differences may explain variation in female dewlap size. They also note that, among Caribbean anoles, females of species that use twigs and large canopy perches seem to have relatively large dewlaps, suggesting that habitat differences may explain variation in female dewlap size.

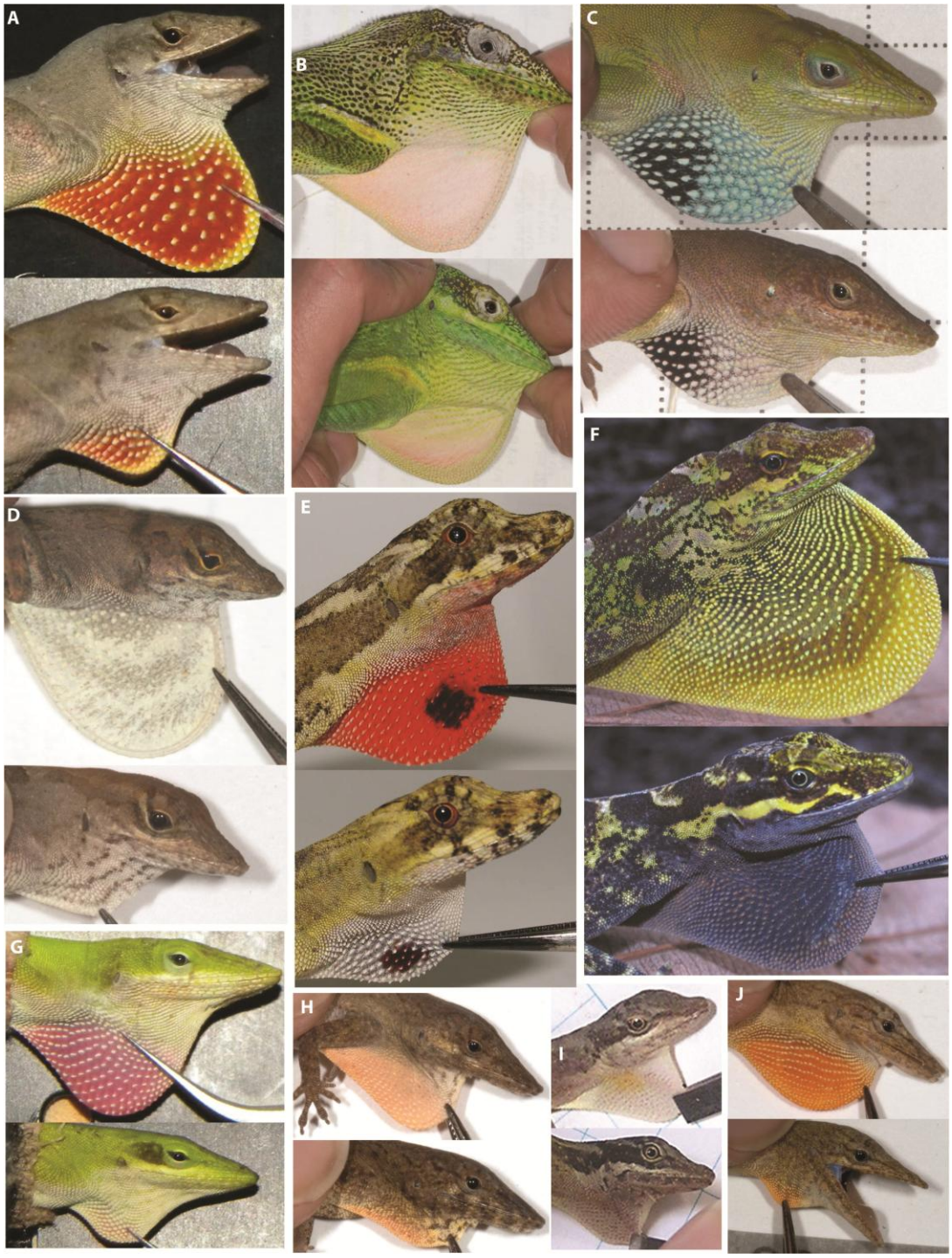

Figure 4.1. Males (top) and females (bottom) of ten species of *Anolis*, demonstrating the range of male and female dewlap sizes. Species are as follow: (A) *A. sagrei*, (B) *A. equestris* (DLM), (C) *A. chlorocyanus* (DLM), (D) *A. homolechis* (DLM), (E) *A. lyra* (FA), (F) *A. fitchi* (FA), (G) *A. carolinensis*, (H) A. *angusticeps* (DLM), (I) *A. limifrons*, (J) *A. garridoi* (DLM). Note the striking contrast in males and female dewlap color for *A. lyra* and *A. fitchi*. Photos were contributed by Luke Mahler (DLM) and Fernando Ayala (FA).

We set out to investigate the factors that influence the evolution of female dewlap size by 1) characterizing the phylogenetic distribution of enlarged female dewlaps in *Anolis* and, 2) using comparative methods to look for correlated evolution between female dewlap size and several morphological and ecological variables.

We tested the predictions of four hypotheses: the *genetic byproduct* hypothesis, which predicts that male and female dewlap size will be correlated, the *social selection* hypothesis, which predicts a correlation between female dewlap size and traits that reflect the mating system, such as sexual size dimorphism (Stamps 1983; Ord et al., 2001; but see discussion in Butler et al., 2000 and Losos, 2010); the *species recognition* hypothesis, which predicts that the female dewlap will be reduced in single-species communities as a consequence of relaxed selection (for a review of trait loss in response to relaxed selection, see Lahti et al., 2009); and the *habitat difference* hypothesis, which predicts a correlation between dewlap size and microhabitat use. It is important to note that these four hypotheses are not mutually exclusive, and that no one hypothesis will necessarily explain female ornament evolution in all species. These hypotheses are summarized in Table 4.1.

Table 4.1. Hypotheses, predictions, and results for four hypotheses proposed to explain variation in female dewlap size.

Hypothesis	Prediction for Female Dewlap	Statistical support
Genetic byproduct	Positive correlation with male dewlap size	χ^2: 55.02, df = 3, p = 0.30
Social selection	Negative correlation with SSD	F: 6.10, df = 33, p = 0.01
Species recognition	Larger in multi-species communities	χ^2: 0.85, df = 3, p = 0.83
Sensory drive	correlation with primary habitat	χ^2: 69.42, df = 3,p < 0.001

Materials and Methods

Data Collection

We compiled data on 228 species from the *Anolis* Handlist (Williams et al., 1995), primary literature, specimens from the Museum of Comparative Zoology and specimens from the personal collection of Steve Poe. We recorded the sizes of the male and female dewlap relative to body size (0 = no extensible dewlap, 1 = posterior edge of dewlap reaches shoulder or is anterior to it, 2 = posterior edge of dewlap is posterior to shoulder), sexual size dimorphism (SSD: log male/female SVL, following Butler et al., 2000), and community composition (1 = species occurs on multispecies island or mainland, 0 = solitary species on an island). Dewlap size is likely an imperfect correlate of how conspicuous a display will be in nature, since color, pattern, habitat, and the motion component of a display all contribute to how a signal is perceived. However we deemed size an appropriate variable for this study because it is the display component that is easiest to measure, it is available for the broadest set of species, and it has been shown in other taxa to correlate well will the conspicuousness of a display (Andersson, 1994). Ninety-three species from the Greater Antilles were also categorized by primary habitat, as reflected by ecomorph classification (crown-giant, grass-bush, trunk-crown, trunk-ground, trunk, and twig: assignments follow Losos, 2009). Habitat use by the six ecomorphs are primarily defined by two variables, perch height and perch diamters. In general, crown-giant anoles use perches that are high and broad, grass-bush anoles use perches that are low and narrow, trunk-crown anoles use a broad range or perch heights and favor medium to narrow perch diameters, trunk-ground anoles use perches that are low and broad, trunk anoles use broad perches over a range of heights, and twig anoles use very narrow perches over a range of perch heights.

Phylogenetic trees

We used maximum parsimony (PAUP; Swofford, 2002) to estimate phylogenetic relationships for 233 species of *Anolis* and eight outgroups using 1673 parsimony-informative characters from DNA sequences (Nicholson et al., 2005) and morphology (Poe, 2004; and unpubl.). This analysis resulted in 6240 equally parsimonious trees. Reliable branch lengths were not available due to uneven coverage of the datasets (i.e., many species were only scored for 50 characters of external morphology); therefore we set all branch lengths to one, corresponding to a speciational model of phenotypic evolution. We generated a 50% majority-rule consensus tree generated using the CONSENSE package in Phylip (Felsenstein, 2005), and traced the character history of the female dewlap on this tree using Mesquite (Figure 4.2: Maddison and Maddison, 2004).

Comparative analysis

We measured phylogentic signal using Pagel's λ to determine if phylogenetic correction was necessary for our analyses (Pagel, 1999; Freckleton et al., 2002; Revell et al., 2008). When λ is equal to 1 related species are similar as expected under a Brownian motion model of evolution, while a value of 0 indicates that the data lack phylogenetic structure. High values of λ indicate that species are similar in proportion to their shared history, indicating that statistical tests that treat species as independent data points would be inappropriate. We used the program BayesTraits to calculate the maximum-likelihood value of λ for both male and female dewlap size, and used a likelihood ratio test to determine whether this value differed significantly from 0, thereby necessitating phylogenetic correction (Pagel and Meade, 2006).

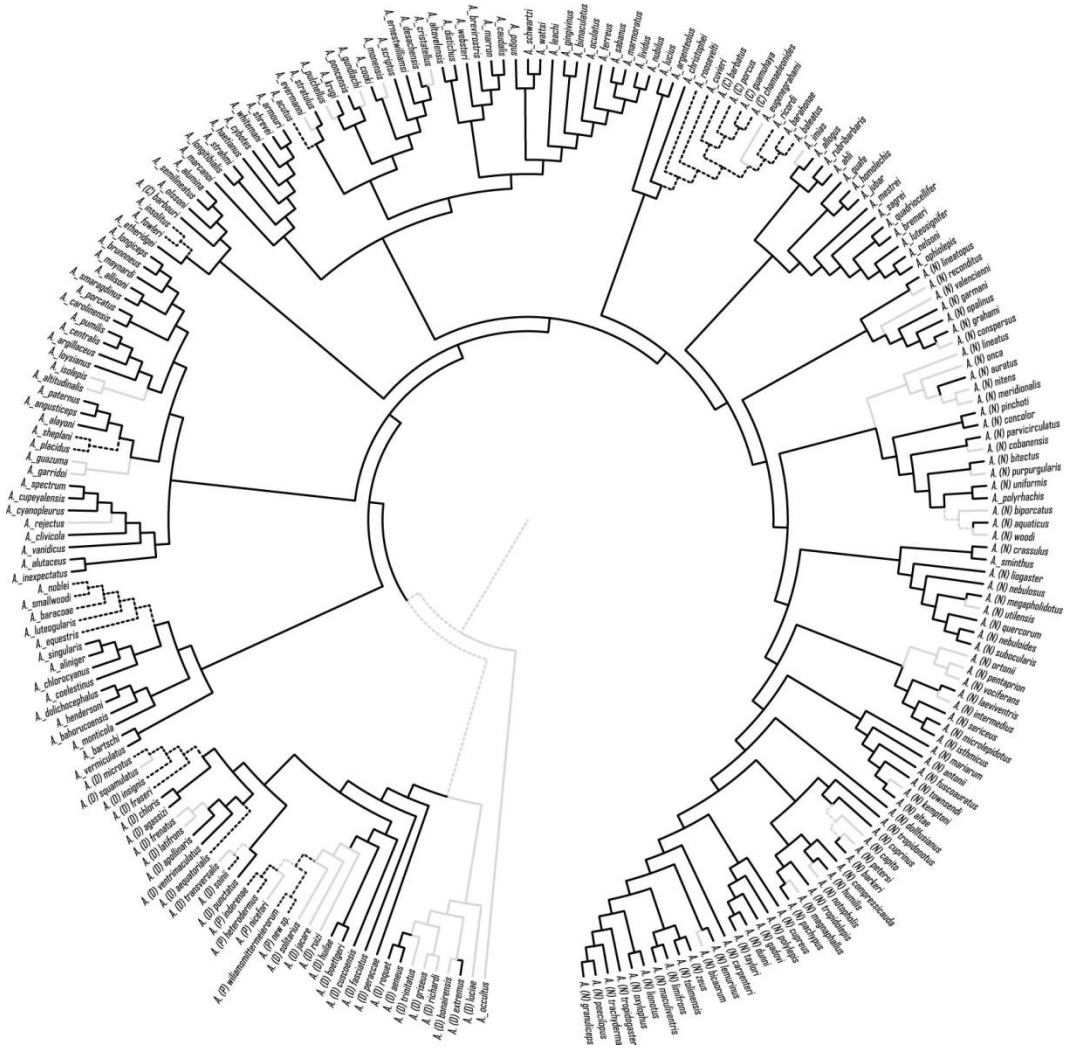

Figure 4.2. A 50% majority-rule consensus tree of 6240 equally parsimonious trees showing a reconstruction of female dewlap size. Dotted black branches indicate a large dewlap (posterior margin extends beyond the shoulder), grey branches indicate a small dewlap (posterior margin at or anterior to the shoulder), and black branches indicate the absence of a dewlap. Taxa for which data is missing, or where the character-state reconstruction is ambiguous, are shown as a dotted grey line. There are several groups within *Anolis* that are recognized as distinct clades and which are sometimes referred to as different genera. Where applicable, these are indicated in parentheses as follows: (C) Chamaeleolis, (D) Dactyloa, (P) Phenacosaurus, and (N) Norops.

Both male and female dewlap size were coded as ordinal data, although the underlying trait is continuous. Most comparative methods require data that are continuous (Felsenstein, 1985; Garland et al., 1992; Martins, 1994; Martins and Hansen, 1997), binary (Pagel and Meade,

2006), or categorical (Garland et al., 1993), and may be inappropriate for ordinal data. Therefore, to estimate the significance of our results while controlling for phylogenetic relatedness, we conducted standard statistical tests, and compared the outcome to a null distribution generated using phylogenetically simulated traits with the same properties as our empirical data (as in Garland et al. 1993).

To create simulated null distributions, we began by simulating continuous data using Brownian motion on our consensus tree with Mesquite (Maddison and Maddison, 2004). We then transformed each continuous variable to an ordinal variable by ranking the values of the variable and assigning each animal to a category based on rank, proportional to the original data (for example, if there were 12 species with "large" male dewlaps, the twelve largest simulated values would be assigned "large"). We generated 1000 values with the same ratio of large:small:absent as our female dewlap data and 1000 values with the same ratios as our male dewlap data. We used these simulated data to construct null distributions with which to compare our actual dewlap data.

We tested the prediction of the genetic byproduct hypothesis by comparing the observed patterns of male and female dewlap association to predicted patterns under correlation of male and female dewlap size. The genetic byproduct hypothesis predicts that the combinations large/large (for male/female dewlap size), small/small, and absent/absent should be especially common and all patterns of unequal-size dewlaps (e.g., large/small, small/large) should be equally uncommon. We used simulated values for male dewlap size, generated as described above, to calculate the average value for each cell in a 3x3 matrix describing all possible combinations of male and female dewlap size (see supplementary materials for matrix). We then

used these values as our expected values to calculate a χ^2 value and estimated the probability of this value with three degrees of freedom.

We also used our species data to estimate the number of evolutionary transitions between four states relating to dewlap size dimorphism between the sexes: monomorphic (large dewlap in both sexes), monomorphic (small dewlap in both sexes), moderately dimorphic, and highly dimorphic character states on our tree. To do this we sampled 100 equally parsimonious reconstructions of ancestral character states generated in Mesquite (Maddison and Maddison, 2004) and took the average number of each type of transition across this group. The genetic byproduct hypothesis makes two predictions about the patterns this data should show. First, when large male ornaments evolve large female ornaments should as well, therefore transitions between monomorphic (both small) to monomorphic (both large) should be more common than transitions from monomorphic (both small) to either moderate or high dimorphism. Second, sexual dimorphism should evolve slowly after the appearance of large ornaments in both sexes, therefore transitions from monomorphic (large) to moderate dimorphism and from moderate dimorphism to high dimorphism should be more common than the reverse.

A phylogenetic Chi-square test was also used to test the species recognition hypothesis, in this case by estimating the expected values in a 2x3 matrix representing female dewlap sized in single-species or multi-species communities, and the habitat difference hypothesis, using data simulated for the 93 species readily assigned to a habitat category.

To test the social selection hypothesis we used a modified version of a phylogenetic ANOVA (Garland et al., 1993), with female dewlap size as the categorical variable and SSD was the continuous variable under consideration. To assess significance while controlling for phylogentic relatedness, we also computed the F statistic for an ANOVA of our 1000 simulated ordinal

female dewlap variables vs. SSD and used this as our null distribution to determine the critical value of F at p=0.05.

Results

For both male and female dewlap size, λ was significantly greater than 0; in fact in both cases the maximum-likelihood estimate was greater than 1 (female dewlap: LR 133.15, $p < 0.01$; male dewlap: LR 13.28, $p < 0.01$). This result indicates that significant phylogenetic structure is present in the data and phylogentic correction is warranted.

The social selection hypothesis was supported by a significant correlation between female dewlap size and SSD (Figure 4.3: $F = 6.1$, $df = 33$, $p = 0.01$, Table 4.1). Species where the female dewlap was small or absent showed a wide range of values for SSD, however SSD was reduced in species in which the female dewlap is large.

The habitat difference hypothesis was supported by the observation that female dewlap size differed significantly among habitat specialists (Figure 4.4: $\chi^2 = 69.42$, $df = 3$, $p < 0.001$, Table 4.1). Giant anoles living in the canopy and on twigs were much more likely to exhibit large female ornaments than anoles using grass-bush, trunk-crown, and trunk-ground habitats. Among trunk anoles, the female dewlap was universally absent.

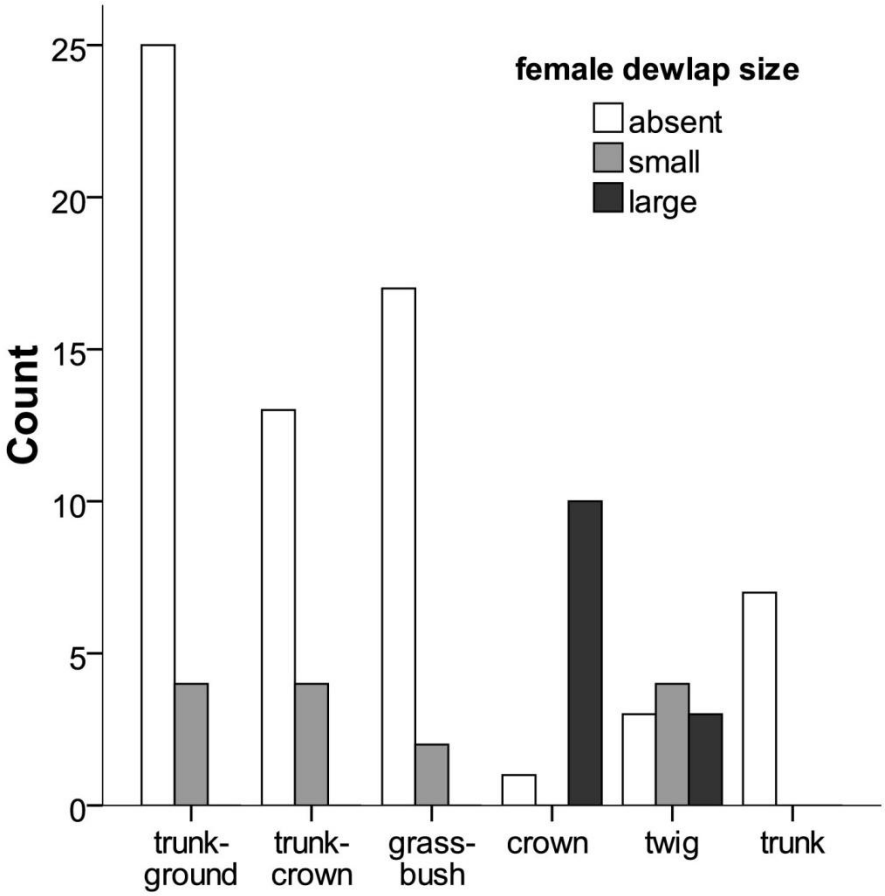

Figure 4.3. Among 93 species easily classified by primary habitat, habitat specialists differ significantly in female dewlap size (ANOVA: F: 6.10, df = 33, p = 0.01). The largest dewlaps (in black) occur exclusively in crown-giant and twig anoles.

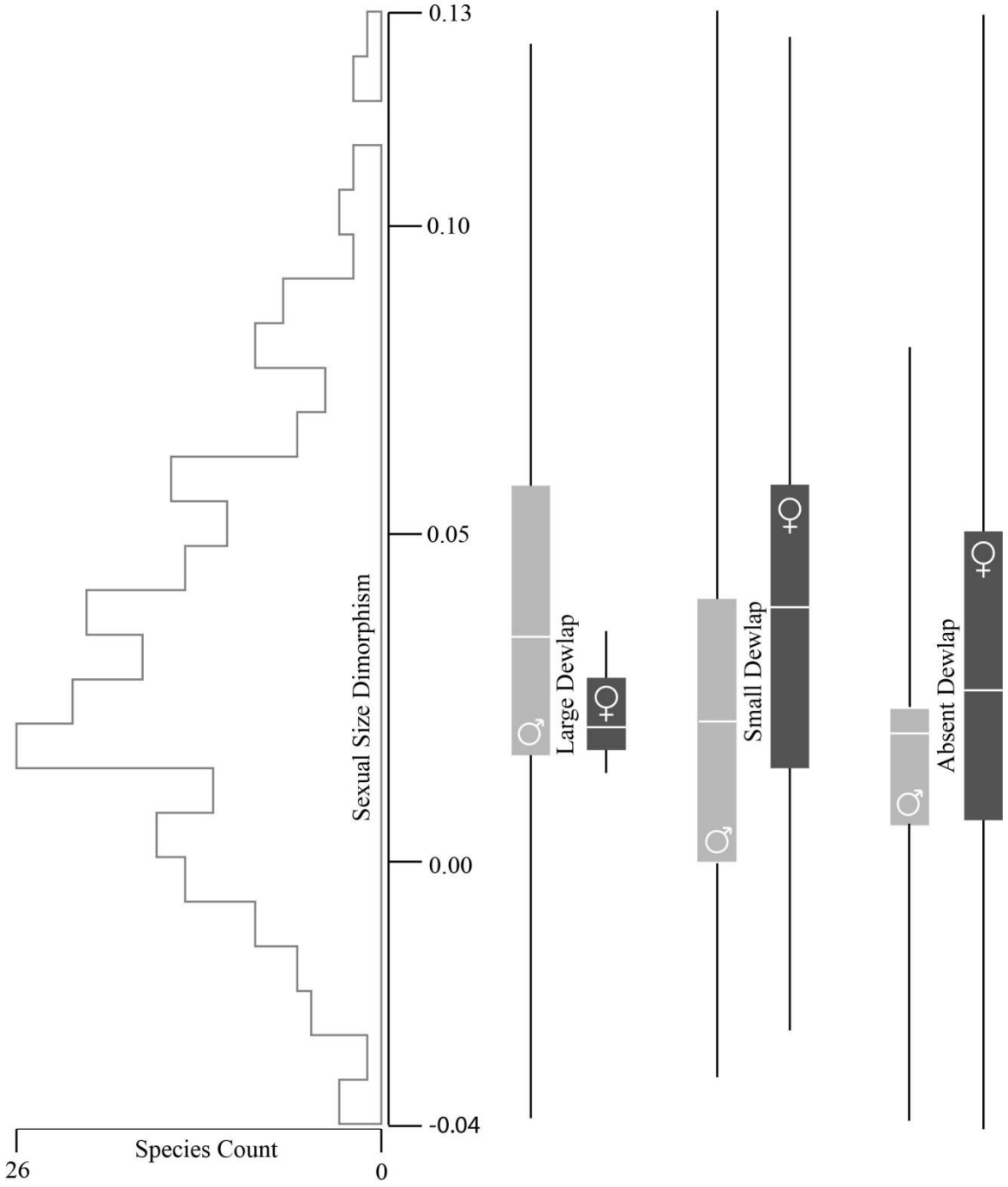

Figure 4.4. The distribution of SSD across all anoles in this study is shown to the left. The distribution of SSD is shown for species with large, small, and absent male dewlaps (light grey) and large, small, or absent female dewlaps (dark grey). Colored bars show the interquartile range, the gap in the bar occurs at the mean value, and whiskers show the 95% confidence interval for each group. Note that, while most groups span the entire range of values of SSD, species for which the female dewlap is large show a low degree of slightly male-biased SSD.

We found no support for either the genetic byproduct hypothesis or the species recognition hypothesis. Female dewlap size and male dewlap size were not significantly correlated (χ^2 = 55.20, df = 33, p = 0.30), and transitions between monomorphic and dimorphic states were common in all directions, with transitions from a higly dimorphic state to reduced dimorphism being especially common (Table 4.2). There was no difference in female dewlap size between single species and multispecies communities (χ^2 = 0.85, df = 3, p = 0.83).

Table 4.2. Average number of evolutionary transitions between four character states relating to dewlap size dimorphism between the sexes: monomorphic (both large), monomorphic (both small), moderately dimorphic (either Large/small or small/absent), and highly dimorphic (Large/absent). Rows indicate the original character state; columns indicate the post-transition state. The number of transitions was estimated as the average over 100 equally parsimonious ancestor state reconstructions

End state: / **Begin state:**	**Monomorphic (both large)**	**Monomorphic (both small)**	**Moderately dimorphic**	**Highly dimorphic**
Monomorphic (large)		0.58	2.72	1.42
Monomorphic (small)	0.9		0.5	0.4
Moderately dimorphic	6.46	1.42		11.8
Highly dimorphic	13.02	11.6	26.18	

Discussion

Despite decades of study on the evolution and function of male ornaments, the evolution of female ornaments is relatively unstudied, and the factors that explain female ornaments remain unclear (Amundsen 2000a, 2000b; Amundsen and Pärn, 2006; Ord and Stuart-Fox, 2006). We tested four hypotheses to explain the size of an ornament, variably expressed in females, the *Anolis* dewlap, using comparative methods and a phylogeny of 228 species. We found evidence of correlated evolution between female dewlap size and SSD, and between female dewlap size and habitat use. We did not find a relationship between male and female dewlap size or a pattern in the transitions from monomorphism to dimorphism, and there was no significant difference in female dewlap size between species living in single and multi-species communities. Taken together, these results support a role for habitat differences and social selection in the evolution of female ornament size in *Anolis,* but reject the genetic byproduct hypothesis and the species recognition hypothesis.

Habitat Differences

Habitat differences may drive differences in ornament size via several mechanisms, including sensory drive, predation, or social selection. We found a strong correlation between female dewlap size and habitat use. In particular, giant anoles inhabiting the tree crown and twigs have large dewlaps relative to other ecomorphs (Figure 4.4). Dewlap size might be enlarged in females of these species for a variety of reasons, and the reasons may differ for each ecomorph.

One possible explanation for this pattern is the sensory drive hypothesis - that differences in the visual properties of a habitat may affect what constitutes a cryptic or conspicuous signal, especially if the sensory systems of the signal recipient have evolved in response to habitat differences. It seems likely that different ecomorphs inhabit different visual habitats. Twig anoles

and crown-giants may live in relatively complex habitats compared with other ecomorphs – crown specialists near the tops of trees and twig anoles in dense vegetation underneath the surface canopy (Williams, 1983; Butler et al. 2000). Recent work examining the motion component of male *Anolis* displays in arboreal habitat has shown that high levels of background movement in arboreal habitat favor more exaggerated displays (Ord et al., 2007). Likewise, the density of vegetation in the habitat of twig and crown giant anoles may require large dewlaps to command the attention of conspecifics through the vegetation. Large dewlaps might be more visible in dense habitats because they are less likely to be completely obscured by intervening leaves and branches. This could be studied directly by characterizing the degree of visual "clutter" in display sited used by different species or by direct behavioral tests of signal efficacy through a complex habitat matrix.

Alternatively, females in these habitats may be able to afford larger ornaments because predation pressure is reduced in less “open” habitats (Stuart-Fox and Ord, 2004; Ord and Stuart-Fox, 2006). This explanation would invoke direct natural selection instead of sensory drive as the cause of signal differences between environments.

Variation in population density may also explain the relationship between female dewlap size and habitat use, and could provide a link between social selection and habitat differences. For example many crown and twig anoles occur at relatively low densities (Luke Mahler, pers. comm.; pers. obs.) and the average distance between signaler and receiver may be relatively far. Indeed, some of the rarest anoles known, including *A. fowleri*, species formerly assigned to the genus *Chamaleolis*, and the elusive mainland alpha anoles (*sensu* Etheridge, 1959), have markedly large female dewlaps. This potential difficulty in locating mates or rivals may necessitate larger signaling structures in both sexes. Lower population densities may also relate

to differences in social structure or mating behavior (Butler et al., 2000). For example, female competition for mates may be exaggerated when conspecifics of either sex are seldom encountered. Further observation of the behavior of rare anoles will be necessary to distinguish between these alternatives.

Although we found a strong relationship between female dewlap size and habitat use, we only tested this hypothesis for 93 species belonging to well-characterized Caribbean habitat specialists. Many species for which the female dewlap is medium or large occur on the mainland (e.g., *A. insignis, A. aequatorialis*), and data on habitat use in these species are scant. More studies on the habitat use and behavior of mainland anoles would provide an opportunity to explore the generality of the correlation between habitat and female ornament size found within the subset of species that we examined.

Furthermore, SSD is known to differ between different ecomorph categories, therefore it is possible that the observed pattern is the result of differences in SSD rather than differences in ecomorph, as we discuss below.

Social Selection

The social selection hypothesis posits that differences in female ornaments are the result of female social interactions, such as sexual selection on females by males or female competition for territories or resources. We found a significant correlation between female dewlap size and SSD: species for which the female dewlap is large showed reduced dimorphism, where the sexes are near to equal in size, while species for which the dewlap was small or absent exhibited a wide range of SSD, including species where both the male and the female are the larger sex (Figure 4.3). In previous work on signal complexity and sexual dichromatism in lizards (Ord et al., 2001; Stuart-Fox and Ord, 2004), male ornamentation was shown to be exaggerated in

species with a high degree of SSD, presumably because both ornament size and male body size are under sexual selection. SSD may also correlate with selection on females, either because females may be directly under sexual selection by males, or because sexual selection on males is in part a function of the spatial and temporal distribution of receptive females - in particular, large body size is likely to be favored in males when reproductive females are spatially or temporally clustered such that a single dominant male can monopolize reproduction within a cluster (Emlen and Oring, 1977).

In *Anolis,* SSD reflects both sexual selection and niche partitioning between the sexes (Schoener 1967; Butler et al., 2000); thus it is unclear how to interpret the observed relationship. If SSD reflects niche partitioning between the sexes, perhaps the correlation between female dewlap size and SSD reflects some aspect of female territorial behavior related to habitat use. Direct data on female competition for resources like food, territory, or nest sites would be illuminating. A relationship between both male and female ornamentation and the strength of sexual selection remains an intriguing possibility, but a better proxy for sexual selection than SSD is necessary to test this hypothesis.

Genetic byproduct

We did not find a significant correlation between female dewlap size and male dewlap size, nor did we find any indication of a prevalence of transitions from monomorphic to dimorphic dewlap sizes (Table 4.2). In fact, transitions from high dimorphism to reduced dimorphism or monomorphism were more common than the reverse. This result differs from previous research on ornament evolution in agamid lizards (Ord and Stuart-Fox, 2006; Stuart-Fox and Ord, 2004), where parsimony reconstructions of ornament evolution showed congruence between the origins of male and female ornaments. However these studies also showed that male and female

ornaments could become decoupled by ornament loss in one sex. Studies of the genetic basis of sexual dimorphism in birds and other vertebrates, for example, show that gene expression can be limited to one sex by modifier genes or regulatory elements that either up-regulate gene expresssion in one sex or supress gene expression in the other sex (Coyne et al., 2007).

While male and female ornaments undeniably share a genetic basis in *Anolis*, it appears that selection has been able to shape the sexes independently. It is worth noting that, although male and female dewlap size were not correlated according to our data, female dewlap size was always equal to or smaller than the dewlap of conspecific males. This pattern merits further investigation.

Species Recognition

We found no correlation between community richness and female or male dewlap size. While dewlap size may function in species recognition under some circumstances, the evidence suggests that this is not the primary function of large dewlaps in female *Anolis*. However, our data on community richness were simple (all communities with more than one species were lumped together) and better data on community complexity might produce a different result. Nonetheless, to the extent that the dewlap is important to species recognition, we suggest that color or pattern rather than size may be the appropriate trait to examine (see VanHooydonck et al., 2008 for an intraspecific study on this topic).

Caveats and Future Directions

Our work on the evolution of the female dewlap could be improved by using continuous data on dewlap size, a more comprehensive phylogeny with realistic branch length estimates, and direct information on ecological variables such as female competition for mates and territories.

In spite of these limitations, we were able to find a strong correlation between female dewlap size and ecomorph type, and between female dewlap size and SSD.

Future work on female ornaments should involve the integration of behavioral observations, direct experimental tests of ornament use in nature, and comparative tests such as those in this paper. It will be especially important to determine how and when females use their dewlaps under natural conditions. Useful studies would include observation of habitat use by females in the Caribbean and of both sexes on the mainland, observation of female territorial and mating behavior, and experimental manipulation of dewlap size or color to determine the importance of these characters. Those species where dewlap color is sexually dimorphic may prove particularly helpful for such studies, since divergent selection on males and females may be particularly potent in these species.

Chapter 5

Size-assortative pairing and social monogamy in a neotropical lizard, *Anolis limifrons*[2]

Abstract

Social monogamy, the formation of stable male-female pairs, is uncommon among reptiles and is particularly rare among squamates, where only a handful of cases have been reported. Only one case of persistant pair formation has ever been reported in anoles, for *A. limifrons*, at a single site in Costa Rica. Detailed studies of *A. limifrons* at other sites, however, have not shown evidence of pair formation. I revisited the site where pairing was originally reported to observe pair behavior in this species and to measure morphological traits of paired and unpaired animals. I confirmed that male-female pairs are commonly encountered in the wild, though a smaller proportion of the animals observed in this study were found in pairs than previously reported. I also found evidence for size-assortative pairing; larger males tended to be found with larger females and smaller males were found with smaller females. I did not find any differences in the morphology of paired and unpaired animals. Although social monogamy has not been widely reported in squamates, I suggest that more examples of this phenomenon will be described as the social behaviors of poorly known species are increasingly subject to study.

Key words: *Anolis limifrons*, mating system, assortative mating, pair formation, monogamy

[2] Reprinted from: Harrison, A. (2013). Size-assortative pairing and social monogamy in a Neotropical lizard, Anolis limifrons (Squamata: Polychrotidae). *Breviora*, 534: 1-9.

Introduction

Social monogamy, the persistent association between an adult male and an adult female during the breeding season, is relatively common among birds and mammals (Wittenberger and Tilson, 1980), although genetic or mating monogamy is more rare (Petrie and Kempenaers, 1998). Among reptiles however, social monogamy and pair formation are very rare (Uller and Olsson, 2008), and genetic fidelity is almost unknown. Squamates in particular are usually both socially and genetically promiscuous (Bull, 2000; Uller and Olsson, 2008).

There are a few notable exceptions to this generalization (Bull, 2000). The best known case is that of the sleepy lizard, *Tiliqua rugosa*, a long-lived skink native to southern and central Australia (Bull, 2000). This species is active through spring and early summer, when it forages on vegetation, eggs, nestlings, and carrion across a broad home range that can overlap with the home range of other individuals of both sexes. Individuals form stable pairs that appear to be socially and genetically monogamous, both within a single breeding season and across multiple breeding seasons (Bull, 2000). Paired animals are often observed in close proximity to each other, and when they are experimentally separated, both males and females actively work to relocate their partners using chemosensory cues (Bull et al., 1993). Pairs of animals can persist over several breeding seasons; one a pair was observed together over ten consecutive years (Bull, 1994; Bull et al., 1998). However, even in this species not all animals are observed exclusively in pairs: 40% of females and 18% of males were found with more than one partner in a season during radio-tracking surveys (Bull et al., 1998). It appears that males that pursue a polygynous strategy do not benefit: females paired with polygamous males were more likely to have multiple paternity litters as revealed by microsatellite paternity analyses (Bull et al., 1998). It is unclear whether pair formation is equally beneficial to females and if so, how.

Australian skinks in the genus *Egernia* have also been shown to form socially monogamous pairs in nature (*E. whitii*: Chapple and Keogh, 2005; *E. stokesii*: Gardner et al., 2002; *E. cunninghami*: Stow and Sunnucks, 2004). In *E. saxatilis*, not only do males and females form monogamous pairs, they live in close proximity to their subadult offspring in a situation that mirrors the 'nuclear family' found in other vertebrates (O'Connor and Shine, 2003). The Tasmanian snow skink, *Niveoscincus microlepidotus*, also forms pairs that persist, on average, for 29 days during the breeding season (Olsson and Shine, 1998). Aside from skinks, at least two species of Chameleon, *Chamaeleo hoehnelii* and *C. jacksoni*, have also been observed in stable pairsin the field (Toxopeus et al., 1988). In *C. hoehnelii*, pairs persisted for an average of 85 days, and 30-40% of all animals were observed in pairs. In *C. jacksoni*, pairs persisted for an average of 63 days. About half of females were observed in pairs while about a third of males were paired.

Despite these reports of pairing behavior in a handful of species, social monogamy is thought to be extremely rare in squamates. Numerous studies of a wide variety of species support the notion that most squamates are polygamous (reviewed in Stamps, 1983).

Why monogamy?

Little is known about why some species of lizards associate in pairs while most do not (Bull, 2000). Three non-mutually exclusive hypotheses may account for socialmonogamy across animal taxa: 1) males remain with females to provide parental care (e.g. food or protection); 2) males guard females because the potential benefit of matings with other females is outweighed by the loss of paternity if other males mate with the focal female); and 3) an individual may remain in the presence of a mate because it benefits directly from their presence (e.g. male may fend off harassment by other males, or both partners may improve the chance of spotting

predators; Bull, 2000). Parental care is typically rudimentary or absent in squamates (Gans, 1996); therefore parental care is unlikely to explain most examples of social monogamy in lizards. A more likely explanation is that either males, females, or both directly benefit from pairing with a single partner. One plausible scenario is that monogamy evolves as a consequence of mate guarding in species where it is difficult for males to successfully defend multiple females, such as when females occur at low densities and occupy a broad home range (Emlen and Oring, 1977; Bull, 2000). Alternately, social monogamy may evolve when the direct benefits of pairing are especially high, such as when predation risk is substantially reduced by an extra set of eyes.

When monogamy does evolve, the process by which individuals form pairs becomes highly important because an individual's fitness may be closely tied to the quality of its mate. Each individual should therefore strive to pair with the highest quality mates. A common pattern in animals is for the largest males to pair with the largest females and smaller males to pair with smaller females, a pattern known as size-assortative mating (SAM; Crespi, 1989). SAM can be a product of three processes: mutual mate choice for large body size (e.g. beetles: Harari et al, 1999; and spiders: Masumoto, 1999), physical constraints (e.g. beetles: Brown, 1993; and fish: Bisazza, 1997), or mate availability − when, for some reason, individuals that are similar in size are more likely to encounter each other and pair due to chance(e.g. limpets: Pal et al., 2006). One way that mate availability could lead to a pattern of size assortative mating would be that, on reaching sexual maturity, an animal pairs with the first unpaired, sexually receptive animal they encounter. Older and larger animals are likely to be paired already and smaller animals are not yet sexually mature, so they are most likely to pair with an animal of similar size.

It may be possible to differentiate among these processes based on differences between paired and unpaired animals. For example, if one sex is more abundant than the other, paired animals should be larger than unpaired animals in the more abundant sex if mutual mate choice for body size is operating. In contrast, if physical contraints are responsible for SAM then the size of unpaired animals should be related to the size distribution of potential mates - large animals may remain unpaired if large mates are rare. Studying SAM can therefore provide insight onto the process of pair formation. In addition, when SAM occurs it can also have important implications for social behavior, population genetics, and even potentially speciation if assortative mating produces a division in the gene pool (Crespi, 1989; Kawecki, 1998; Nagel and Schluter, 1998; Harari et al., 1999; Bessa-Gomes et al., 2003).

Pairing behavior in A. limifrons

A surprising candidate for pair formation and SAM in lizards is a Costa Rican population of *Anolis limifrons*, a slender arboreal lizard that is abundant in a variety of habitats from southern Mexico to Panama (Savage, 2002). This is very unusual behavior for an anole, a genus in which males typically gain access to multiple females by defending a territory and excluding other males (Trivers, 1976; Andrews, 1985; Jenssen and Nunez, 1998; Losos, 2009).

Pair formation in *A. limifrons* was first reported by Talbot (1979), who found that 70 % of adults were found in male-female pairs, that is, a single male and a single female were found within 2m of each other with no other lizards present,. Mark-recapture data showed that these pairs persisted for 4-6 months, approximately the adult lifespan for this species. Detailed observations of pair behavior revealed that individuals in these pairs display to each other frequently (Figure 5.1) and move in tandem for distances up to 20 meters (Talbot, 1979).

Figure 5.1. A male *A. limifrons* displays to a female on an adjacent perch. These animals were observed in close proximity and were interacting. No other lizards were observed in the area, suggesting that these animals form a pair.

Stable pairs have not been observed in other populations of *A. limifrons* despite extensive study; in fact other populations demonstrate typical resource-defense polygyny (Andrews and Rand, 1983; Andrews and Stamps, 1994). Pair formation has also never been studied in other anoles, though it has been suggested for two species beside *A. limifrons* based on the proximity of sleeping males and females (*A. occultus*: Gorman, 1980, and *A. cuvieri*: Ríos-Lopez and Puente-Colon, 2007). This study had three objectives: to observe pairs of *A. limifrons* at the site where Talbot collected his data, to determine if such pairs demonstrate size-assortative pairing and to compare the morphology of paired and unpaired animals.

Materials And Methods

Field Data

Field data were collected during a 17 day period from 19 April - 5 May 2007 at La Selva Biological Reserve in the Heredia province of Costa Rica between 7:30am and 5:30pm. Individual *A. limifrons* were spotted by walking slowly along established trails while visually surveying vegetation, a standard method for conducting herpetological surveys (Doan, 2003). When an individual was spotted, two observers positioned themselves approximately five meters away from the subject. One observer recorded the behavior of the focal animal while the other scanned the area to identify other nearby individuals. If no additional lizards or displays were observed during the first 15 minutes of observation, the lizard was considered "solitary" and was captured. If the initial lizard displayed, or if another lizard was spotted nearby, the observation was extended to 30 minutes. Following the procedure of Talbot (1979), two lizards were considered a pair if they were observed within two meters of each other, and no other lizards were seen within five meters during the observation period. In most cases, paired males and females were less than half a meter apart. On some occasions, more than one lizard was observed within two meters of the initial animal. In all of these cases, a single female was found in the presence of multiple males; multiple females were never found in such a group. It seems likely that these groups represented a pair and one or more intruders but, because it was impossible to determine which male or males were intruding, these cases were excluded from the final analysis (but see supplementary materials to see how the inclusion of these animals would affect the observed pattern). In some cases, more than one individual was identified during an observation,

but not all lizards were successfully captured. If a lizard was spotted but not captured, no animals from the observation were included in subsequent analysis.

After 30 minutes of observation, attempts were made to capture all lizards by hand or by noose. Each lizard captured was measured, photographed with the dewlap extended, marked with a unique pattern of colored ink dots on the ventral surface, and released at the site of capture. The data recorded for each individual were sex, snout-vent length (svl, a standard variable for estimating body size in lizards, forelimb length, hindlimb length, tail length, and the length, depth and width of the head, to the nearest tenth of a millimeter. Males less than 32mm in svl and females less than 35mm in svl were considered juveniles (following Talbot 1979) and were excluded from further analysis (but see supplementary materials to see how inclusion of these animals would affect the analysis). All measurements were taken with digital calipers by the same individual. The surface area of the dewlap was measured for all males in ImageJ (Abramoff et al., 2004) and scaled by reference to a 1cm grid.

Statistical Analysis

The morphological traits of paired and solitary animals were compared using an analysis of covariance. These traits included svl, fore- and hindlimb length, tail length, head length, width and depth, and dewlap area; body size was included as a covariate in the analysis of all traits except body size. Males and females were treated separately. As no tests approached significance, no correction for multiple tests was used.

The correlation between body size for members of a pair was calculated using Pearson's correlation coefficient. All statistics were calculated in SPSS.

Results

Among 150 animals observed and captured, 40 individuals were found in male-female pairs; 33 males and 15 females were observed alone; 22 males and 8 females were observed in groups of more than two animals; and 22 males were observed in male-male pairs. Ten males were observed with another animal, but the other individual was not captured and the sex could not be definitely assigned. When animals whose pair status could not be assigned are excluded (e.g. animals in groups, male-male pairs, pairs where one animals was not captured), 57.1% of females and 37.7% of males were observed in pairs (44.9% average for both sexes). Individuals in male-female pairs were often interacting during the time that they were observed, although copulation was never witnessed. Visual displays were performed by both males and females (males displayed in 8/20 observations while females displayed in 2/20). Often one animal, typically the male, followed the other up and down a perch, and from one perch to another (5/20 observations), often moving slowly and frequently stopping until the partner was quite close.

During the course of three observations, a male and female in close proximity were approached by a second male who the first male proceeded to chase away. While these males was thus distracted, a third male would suddenly appear and approach the female while, displaying. In one case the female approached the third male and watched him display. In the other two cases, the female retreated from the third male. Copulation was never observed during the course of these intrusions.

Male and female body size were positively correlated for paired animals (Figure 5.2, $R = 0.50$, $p = 0.039$), and these results were qualitatively similar when smaller animals were included, or when animals found in groups with one female and several males were included (see

supplementary materials); however neither body size (Figure 5.2), body dimensions, or dewlap area differed between paired and solitary animals (see supplementary materials).

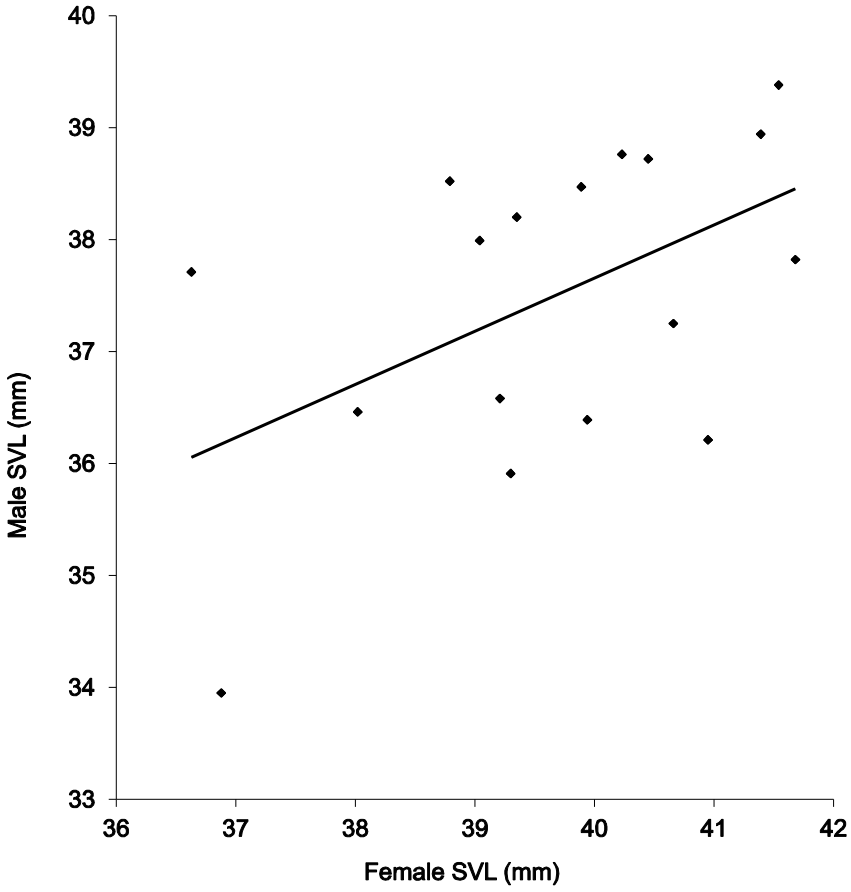

Figure 5.2. Correlation between male and female body size for paired animals, each pair is symbolized by a grey diamond.

Discussion

Pair formation and social monogamy are rarely observed in reptiles, particularly squamates, yet during the course of this study, male-female pairs were observed quite frequently. In most cases, members of a pair were within 50 cm of each other or less, and were frequently observed

displaying to each other. Pairs also showed coordinated movement, in which one animal (usually the male) followed the female vertically or horizontally through the habitat. These observations, combined with Talbot's (1979) observations of pair persistence over 4-6 months, indicate that these animals are not in association by chance - they are in fact associating in pairs. This social behavior is highly atypical for lizards in general and for anoles in particular, which more typically demonstrate resource-defense polygyny (Losos, 2009). This behavior may also be atypical for *A. limifrons,* which has been shown to defend polygynous territories in other parts of its range. These data also show one of the first reptilian examples of size-assortative pairing(SAP). We found no morphological differences between paired and unpaired animals, suggesting that mutual mate choice is an unlikely explanation for SAP. Two other hypotheses to account for SAP, physical contraints and mate availability, were neither supported nor rejected by these data, but remain a fruitful avenue for future research efforts.

Existence and prevalence of pairs

The rates of pairing observed in this study were lower than those observed by Talbot: 45% vs. 70% of all individuals, respectively. The lower percentage in this study may have resulted because some paired individuals may have been observed when their partner was not visible, or pairs may have been excluded from analysis due to the presence of a temporary intruder. Unpaired animals might also have been mistaken for paired animals due to temporary proximity to a member of the opposite sex.

It is also possible that the pairing behavior is not typical of *A. limifrons,* and occurs occasionally at this location due to unknown environmental or social factors. In other well-studied populations of *A. limifrons*, pairing between males and females has never been reported, despite intensive study (Andrews and Rand, 1983; Andrews and Stamps, 1994). Rather, these

populations exhibit a sedentary polygamous mating system based on territory defense; a system typical of anoles. A possible explanation is that *A. limifrons* observed at sites outside La Selva actually belong to a different species, which has diverged in social behavior. Recent morphological work has suggested that *A. limifrons* may actually consist of three or more distinct species(Köhler and Sunyer, 2008). If *A. limifrons* at La Selva comprise a unique lineage, their distinct evolutionary history could have influenced the evolution of their social behavior in a number of ways, via shifts in habitat use, population density, or predation risk, to name a few possibilities.

Another possibility is that *A. limifrons* has a high degree of behavioral plasticity in their mating behavior and that local conditions determine what strategy they pursueThis would be consistent with the polygyny threshold model, which suggests that individual mating decisions may change when the distribution of resources shifts to allow higher densities and/or stable aggregations of females that males can monopolize (Altmann et al., 1977). Although data on forest characteristics were not collected as part of this study, it appeared that pairs occur more frequently in primary forest, whereas clusters of animals were found in great abundance in disturbed habitat. This potential pattern could be a productive direction for future studies.

Characteristics of pairs

Although the data show SAP in *A. limifrons*, I did not find support for the mutual mate choice hypothesis, the physical constraints hypothesis, or the mate availability hypothesis. Paired and unpaired animals did not differ in any of the morphological variables that were considered, including body size, head, limb and tail dimensions, or dewlap area, suggesting that mate choice is not operating on these traits (see supplementary materials for details). Mutual mate choice may be operating on traits that we did not consider, or a pattern of mate choice in the traits that were

examined could have been obscured by the shortcomings of our survey methods or confounding ecological processes that also act on body size, such as differential mortality.

Mating constraints seem unlikely to be responsible for SAM in *A. limifrons*, as pairs were observed that were somewhat size-mismatched (and that deviate substantially from the observed correlation), but I cannot rule out this possibility entirely because physical constraints may only be relevant to the most extreme mismatches and I did not observe all possible size combinations in the population. Captive breeding experiments with highly size-mismatched individuals could be conducted to test this hypothesis explicitly. Likewise, No spatial or temporal discontinuity was observed in the distribution of size classes that would support a mate availability mechanism as an explanation for SAM. However, the spatial distribution of body sizes was not explicitly examined, and the duration of this study was insufficient to uncover temporal patterns of variation in body size. Further studies that explicitly examine the spatial and temporal distribution of body size and paired and unpaired animals may clarify this issue. In short, the process that is responsible for the pattern of SAP in *A. limifrons* remains obscure; uncovering the process of pair formation in this species will require substantial future efforts.

More questions than answers

The existence of socially monogamous pairs in *A. limifrons* does not necessarily imply that these pairs are also genetically monogamous. Molecular studies of parentage will be necessary to characterize the genetics of this unusual social behavior. Moreover, we still have little conception of how pairs are formed in the wild, or what benefits accrue to pair members. There is a wealth of opportunity for future studies in the field and in the lab on these questions.

The unusual behavior of *A. limifrons* also begs the question: Could social monogamy be more common in lizards than previously thought? The behavior of most species of anole is

poorly known from observations in the field – indeed, the behavior of very few lizard species has been studied in the wild. Species that have been overlooked in previous studies are precisely the ones that are predicted to exhibit social or genetic monogamy; for example species with wide home ranges without established territories or territorial behavior, low densities, and cryptic appearance and habits (Emlen and Oring, 1977). The unusual social behavior of *A. limifrons* described in this study, combined with the fact that it is relatively abundant, easily observed, and geographically widespread could make this a useful species for future studies of pairing behavior in squamates.

Chapter 6

Correlated evolution of microhabitat, morphology, and behavior in West Indian *Anolis* lizards: A test of the habitat matrix model

Abstract

The unique environment inhabited by arboreal animals has led to morphological convergence among taxa as distinct as squirrels, lizards, and primates. According to the habitat matrix model (HMM) differences in microhabitat structure drive evolution in morphology, locomotor mode, and habitat use. This should result in divergence of species that occur in structurally different microhabitats; and convergence of species in similar habitats. Although tests of this model have generated important insights into the ecology of arboreal species, generally speaking these tests have left a gap in the habitat-behavior-morphology story by focusing primarily on locomotor performance in lab and field experiments and thus failing to include data on locomotor behavior of undisturbed animals in the wild. To evaluate the HMM, we collected data on undisturbed locomotion, habitat use, and morphology for 31 species of arboreal lizard in the genus *Anolis* and used these data to test nine specific predictions that arise from the HMM. We find strong support for nearly all aspects of this model. Specifically, species that inhabit narrow perches walk often and have short limbs and many lamellae; species on low, dense perches jump often and have long tails and short forelimbs; and species on broad perches run often and have long limbs. The addition of data on locomotion by undisturbed wild animals offers a more direct and compelling case for the HMM than most previous tests. We suggest that the HMM could be applied more widely as a framework for understanding the correlated evolution of functionally linked

morphology and behavior, not only for arboreal species, but for any species where locomotion occurs in three dimensions.

Key words: arboreal locomotion, convergent evolution, *Anolis*, ecomorphology, habitat matrix model

Introduction

Convergent evolution of species living in similar environments is strong evidence of evolutionary adaptation (Harvey & Pagel, 1991; Conway Morris, 2004; Losos, 2011). Arboreal species often evolve convergent locomotor morphologies, presumably because the structure of their habitat poses distinct challenges compared to locomotion on the ground. The habitat matrix model (HMM) posits that the three-dimensional matrix of branches that comprise arboreal habitats place functional constraints on how organisms can move, leading to predictable differences among species in habitat use, locomotor behavior, and morphology (Moermond, 1979a, b; Pounds, 1988). A critical, but underappreciated, aspect of this model is that the predicted relationship between habitat and morphology depends on the locomotor mode (e.g. walking, jumping) that an animal utilizes. For example, the relationship between the width of a branch and the optimal length of an animal's limbs depends on how frequently it walks, runs, or jumps under normal conditions (Moermond, 1979a,b).

The HMM makes several specific predictions about the relationships between habitat, morphology, and behavior (summarized in Table 6.1) based on functional arguments concerning the demands of specific locomotor modes and the conditions under which each mode is optimal (Moermond, 1979a, b; Pounds, 1988). The first type of prediction relates habitat to locomotor

mode. For example, species using perches that are low to the ground and closely spaced will tend to jump among perches (Prediction 1), because jumping between perches provides greater mobility than running or walking along a single perch. The second type of prediction relates locomotor mode to morphology. For instance, species that jump frequently are predicted to have longer tails and shorter forelimbs to control pitch during jumping, combined with longer hindlimbs to propel their leaps (Prediction 2). The sum of the predicted relationship between habitat + behavior and behavior + morphology is a third type of prediction relating habitat to morphology. For example, combining the two predictions described above leads to a third prediction that species using low, dense perches will have longer tails and hindlimbs, and shorter forelimbs (Prediction 3), as a consequence of their frequent jumping.

Table 6.1. Predictions of the Habitat Matrix Model.

	Prediction	Class of prediction	Result
Species that jump frequently			
1	Species using dense, low perches will jump more frequently than they walk or run	**Habitat + behavior**	Low perch height correlated with high jumping frequency in H + B canonical correlation 2 ($R = 0.41$, $p = 0.04$).
2	Species that jump relatively frequently will have longer tails and hindlimbs, and shorter forelimbs	**Behavior + morphology**	Frequent jumping correlated with short forelimbs and long tails in B + M canonical correlation 2 ($R = 0.64$, $p = 0.01$).
3	Species using low, dense perches will have longer tails and hindlimbs, and shorter forelimbs	**Habitat + morphology**	Low perch height not significantly correlated with short forelimbs and long tails in H + M canonical correlation 2, but predicted trend is present ($R = 0.47$, $p = 0.08$).
Species that walk frequently			
4	Species using narrow perches will walk more frequently than they run or jump	**Habitat + behavior**	Narrow perch diameter correlated with high walking frequency in H + B canonical correlation 1 ($R = 0.67$, $p = 0.001$).
5	Species that walk relatively frequently will have shorter limbs and tails and more lamellae	**Behavior + morphology**	High walking frequency correlated with short limbs and tail and more lamellae in B + M canonical correlation 1 ($R = 0.79$, $p < 0.001$).
6	Species using narrow perches will have shorter limbs and tails and more lamellae	**Habitat + morphology**	Narrow perch use correlated with short limbs and tails and more lamellae in H + M canonical correlation 1 ($R = 0.84$, $p < 0.001$).
Species that run frequently			
7	Species using broad perches will run more frequently than they walk or jump	**Habitat + behavior**	Broad perch use correlated with frequent running in B + H canonical correlation 1 ($R = 0.67$, $p = 0.001$).
8	Species that run relatively frequently will have long hindlimbs and forelimbs	**Behavior + morphology**	Frequent running correlated with long forelimbs in B + M canonical correlation 2 ($R = 0.64$, $p = 0.01$).
9	Species using broad perches will have long forelimbs and hindlimbs	**Habitat + morphology**	Broad perch use is significantly correlated with long limbs in H + B canonical correlation 1, ($R = 0.84$, $p < 0.001$).

The predictions of the HMM have proved to be a useful framework for prior studies of arboreal locomotion in a variety of taxa (e.g. primates [Crompton, 1984]; birds [Moreno & Carrascal, 1993]; possums [Schmidt & Lemelin, 2002]; squirrels [Essner, 2007]; and lizards [Vanhooydonk et al., 2000; Herrel et al., 2001; Kohlsdorf et al., 2001; Bickel & Losos, 2002; Goodman et al., 2008]). By and large, however, these studies have not included data on locomotor behavior in nature. Rather, previous studies have primarily investigated the predictions of the HMM from two angles. First, the third type of prediction (habitat + morphology) has been supported by a wide variety of correlational studies (e.g. Herrel et al., 2001; Bickel & Losos, 2002; Essner, 2007; Goodman et al., 2008). For instance, previous work on lizards has shown that habitat use is correlated with many aspects of the phenotype such as body size, limb dimensions, tail length, and movement rate, among others (Williams, 1972, 1983; Losos, 1990b,c; Losos et al., 1998; Beuttell & Losos, 1999, Butler et al., 2000; Butler & Losos, 2002; Harmon et al., 2005; Vanhooeydonk et al., 2006; Losos, 2009).

A second group of studies have used biomechanics to test the functional relationships between morphology, perch type, and locomotor performance (Losos & Irschick 1996; Irschick & Jayne, 1999; Mattingly & Jayne., 2004; Vanhooydonk et al., 2006; Goodman et al., 2008). For example, studies that have tested running performance in the lab have shown that limb length and running speed are correlated, but the advantage of long limbs is more pronounced on broad perches (Irschick & Losos, 1999). On narrow perches, long-limbed lizards are still somewhat faster, but are more likely to slip or stumble, leading to a loss of speed (Losos & Sinervo, 1989; Irschick & Losos, 1999). Furthermore, the maximal speeds of species measured in the lab were found to be good predictors of maximal speeds during simulated predator attacks in the wild (Irschick & Losos, 1998). Jumping ability is also correlated with limb length. Specifically,

species with longer hindlimbs are capable of jumping farther (Losos 1990d; Toro et al., 2006), although it appears that jumps of maximum length are rarely performed in the wild (Irschick & Losos, 1998).

While previous studies such as these provide indirect support for the HMM, the model has seldom been tested directly by incorporating data on the frequency of locomotor modes of undisturbed animals (but see Pounds, 1988; Losos, 1990a for exceptions). The predictions of the HMM depend on the link between locomotor mode and habitat and locomotor mode and morphology. In particular, six of the nine predictions of the model concern locomotor mode outright, while locomotor mode is implicit in the other three. In this sense, major portions of the HMM have been neglected by previous work.

In this study, we set out to test nine predictions of the HMM using the arboreal lizard genus *Anolis*, for which the model was originally developed (Moermond, 1979a,b; Pounds 1988; Losos, 1990a). We combined field data on locomotor behavior and microhabitat use with morphological measurements for 31 species of *Anolis* from the Greater Antilles to look for correlations between sets of variables using a phylogenetically corrected canonical correlation analysis. We find the predictions of the HMM to be largely upheld, especially when locomotory behavior is incorporated.

Materials and Methods

We collected data on the morphology, habitat use, and locomotor mode of adult males from 31 species of *Anolis* lizard from the Greater Antilles (Table 6.2). We then used canonical correlation analysis (CCA) to look for the strongest correlations between sets of variables. Because species cannot be treated as independent data points, we performed our analyses with

phylogenetic correction (pCCA - described in detail below). In total, we performed three canonical correlations: habitat variables vs. behavioral variables, behavioral variables vs. morphological variables, and habitat variables vs. morphological variables. We then examined the correlations that best relate these datasets to determine if they supported the predictions of the HMM.

Table 6.2. Species included in the analysis. Dominant locomotor frequencies are reported.

Island	**Species**	**Preferred locomotor mode**
Cuba	*Anolis allogus*	46% Running and 43% jumping
	A. alutaceus	56% Jumping and 35% walking
	A. angusticeps	66% Walking
	Chamaeleolis chamaeleonides	89% Walking
	A. homolechis	45% Walking and 44% running
	A. loysiana	52% Walking and 41% running
	A. luteogularis	72% Walking
	A. mestrei	45% Walking
	A. porcatus	59% Walking
	A. sagrei	49% Walking and 27% running
	A. vermiculatus	55% Walking
Hispaniola	*A. aliniger*	86% Walking
	A. brevirostris	96% Running
	A. chlorocyanus	70% Walking
	A. christophei	83% Running
	A. cybotes	57% Running
	A. distichus	57% Running
	A. insolitus	81% Walking
	A. olssoni	40% Running and 38% jumping
Jamaica	*A. garmani*	45% Walking and 45% running
	A. grahmi	49% Running and 31% walking
	A. lineatopus	57% Running and 27% jumping
	A. opalinus	70% Running
	A. valencienni	72% Walking
Puerto Rico	*A. cristatellus*	58% Running
	A. evermanni	53% Walking and 31% running
	A. gundlachi	43% Running and 34% walking
	A. krugi	50% Walking
	A. poncencis	44% Walking and 38% running
	A. pulchellus	61% Walking
	A. stratulus	55% Running and 35% walking

Behavior, morphology, and microhabitat use

We collected field data during summer months in 1987-1989 and in 1997. Undisturbed lizards were located for observation by slowly walking through the habitat. We observed lizards for up to 20 min (some lizards disappeared from view before the end of the observation session) and included only individuals watched for at least 5 min and which moved at least five times to exclude animals potentially disturbed by our presence. For each individual, we recorded the total number of movements, as well as the proportion of total movements that were classified as running, jumping, and walking. These numbers are summarized in Table 6.2. We then calculated species' means. For non-Cuban species we also measured perch height and diameter for all perches used during the observation. For the Cuban species adequate comparable perch data were not available, thus only the dimensions of the perch where the lizard was first spotted were used. Both the morphological and microhabitat variables measured in this study have been used extensively in prior ecological and evolutionary studies (e.g. Losos 1990b; Johnson et al., 2008). Data for Jamaican and Puerto Rican species were previously published in Losos (1990a).

Prior to analyses, we transformed all variables to conform to the statistical expectations of the analysis. Running frequency was excluded (because of non-independence among proportions summing to 1). Canonical variates that loaded negatively on both jumping and walking were inferred to reflect a high frequency of running for interpretation of the results. Jumping and walking frequency were arcsine square-root transformed (that is, we computed the inverse sine of the square-root; a common transformation for relative frequency data). Because many morphological traits are highly correlated with size, we removed the effect of size from all

morphological variables (except SVL) by phylogenetically regressing each variable on size and then computing the residuals (Revell, 2009).

Evolutionary correlations among characters

To investigate the relationship between morphology, microhabitat, and locomotor behavior, we used canonical correlation analysis. Canonical correlation analysis is a statistical method in which two sets of orthogonal derived variables are calculated from two sets of original variables such that the correlations between corresponding derived variables are maximized. This method allows us to identify linear functions of each set of variables that have maximum correlation with other such sets (Miles and Ricklefs 1984). The number of correlations generated by this method will be determined by the number of variables in the smaller variable set.

Canonical variables can be interpreted by calculating the correlations between each set of canonical scores and all original traits (Miles & Ricklefs, 1984). These canonical loadings (also called structure coefficients) are included because they can help make the canonical variables more readily interpretable in the original space. Within-set correlations describe the contribution of each trait to its canonical variable; whereas between-set correlation reveal the relation of each trait to the canonical variable in the other set (Miles & Ricklefs 1984).

Like most standard statistical methods, an important assumption of canonical correlation analysis is that our data represent independent draws from a single underlying multivariate normal distribution. When observations are from species related by a phylogeny, this assumption is typically invalid because differing amounts of shared history among the species in the sample create non-zero expected covariances between the observations for different species under most models for the evolutionary process (Felsenstein 1985; Martins & Hansen 1996). In the most common evolutionary process model for continuously distributed characters, Brownian motion,

the statistical dependence amongst the observations at the tips of the tree is exactly proportional to the shared common history from the root to the common ancestor of the tips (Ives & Garland 2000; Rohlf 2001; Revell & Harmon 2008; Revell 2008). Under an assumption of Brownian motion, the statistical dependence of the observations from the tips can thusly be removed from the data matrices using a relatively simple transformation (Rohlf, 2001; Revell & Harrison 2008). The specific linear transformations used are provided in Revell & Harrison (2008) with specific reference to canonical correlation analysis.

This assumption of Brownian motion as evolutionary process can be relaxed somewhat by adding an additional parameter to our model for the covariances between species: λ, initially proposed by Pagel (1999). λ is a multiplier of the covariances between species, and we can identify the joint maximum likelihood estimate of λ that best fits the observations at the tips of the tree. Under this model, the expected covariance between any pair of species relative to their variances is not directly proportional to their common history (as in the typical Brownian motion model), but is a linear function of that history. The value of λ is estimated using maximum likelihood for all traits simultaneously following Freckleton et al. (2002). Since Brownian motion is a special case of the λ model (specifically, one in which $\lambda = 1.0$), we can fit both multivariate Brownian motion and multivariate λ and then pick the best fitting model using standard approaches, such as the likelihood-ratio test.

The behavioral variables included in the canonical correlation analysis include log-transformed total movement rate, and arcsine square-root transformed jumping and walking frequencies. Microhabitat variables included perch height and diameter. Finally, the morphological variables included were snout-vent length (SVL), relative lamella number, and the relative length of the forelimb, hindlimb, and tail. For this study, we obtained our point

estimate of the phylogeny by subsampling the maximum likelihood tree of Nicholson et al. (2005) to include only the 31 species of this study.

We performed all canonical analyses using the maximum likelihood estimates (MLEs) for λ as well as by setting λ to 1.0, which corresponds to a strict assumption of Brownian motion. While the results for both models were qualitatively similar, we only report the results for the MLE (λ) analysis because this model has Brownian motion as a special case and thus makes fewer assumptions about the nature of the evolutionary process by permitting covariances between species that deviate from those expected under Brownian evolution. We performed all analyses using the ape and phytools packages in R (Paradis et al., 2004; Revell, 2012; R core team, 2013).

Results

In all three comparisons (behavior vs. morphology, behavior vs. habitat, and habitat vs. morphology) the first canonical correlation was significant (Table 6.3). For the comparisons of behavior vs. morphology and habitat vs. morphology, the second canonical correlation was also significant (Table 6.3). The correlations between the original characters and the canonical variates (i.e., the structure coefficients) are given in Table 6.4 (behavior vs. morphology), Table 6.5 (behavior vs. habitat), and Table 6.6 (habitat vs. morphology).

Table 6.3. Summary of the phylogenetic canonical correlation analyses for the relationships between: (1) behavior and five morphological variables (morphological variables except SVL were size corrected using separate linear regressions on the phylogenetically transformed data); (2) behavior and two ecological (microhabitat) variables; and (3) morphology and the two microhabitat variables.

				Statistical tests		
	Maximum likelihood λ	Canonical variables	Canonical correlation	χ^2	df	P
Analysis 1: behavior & Habitat	0.44	1	0.67	21.24	8	0.001
		2	0.41	4.97	3	0.04
Analysis 2 : behavior & Morphology	0.26	1	0.79	44.35	20	<0.001
		2	0.64	18.81	12	0.01
		3	0.43	5.18	6	0.08
Analysis 3: habitat & Morphology	1.00	1	0.84	38.07	10	<0.001
		2	0.47	6.64	4	0.08

Table 6.4. Correlations between behavioral and microhabitat variables, and the canonical variables from the phylogenetic canonical analysis of the microhabitat and behavioral variables.

	Canonical variables			
	Behavior		Habitat	
	CV₁	*CV₂*	*CV₁*	*CV₂*
Behavior variables				
Move rate	-0.04	-0.31	-0.02	-0.21
Walks	-0.98	-0.16	-0.65	-0.13
Jumps	-0.11	0.99	-0.05	0.46

Microhabitat variables				
Perch height	-0.32	-0.41	-0.42	-0.89
Perch diameter	0.52	-0.27	0.83	-0.60

Table 6.5. Correlations between morphological and behavioral variables, and the canonical variables from the phylogenetic canonical analysis of the behavioral and morphological variables. These correlations are also sometimes called 'structure coefficients.'

	Canonical variables					
	Morphological			Behavioral		
	CV_1	CV_2	CV_3	CV_1	CV_2	CV_3
Morphological variables						
SVL	0.31	-0.33	0.27	0.26	-0.20	0.21
Lamellae	0.54	0.22	-0.66	0.46	-0.17	-0.25
Forelimb	-0.53	0.60	0.33	-0.44	0.39	0.18
Hindlimb	-0.75	0.03	-0.12	-0.59	0.01	0.02
Tail	-0.43	-0.37	-0.47	-0.29	-0.26	-0.16
Behavioral variables						
Moverate	0.48	0.39	-0.25	0.58	0.60	-0.54
Walk	0.70	-0.36	-0.01	0.86	-0.49	0.20
Jump	-0.30	-0.45	-0.24	-0.34	-0.71	-0.60

Table 6.6. Correlations between morphological and microhabitat variables, and the canonical variables from the phylogenetic canonical analysis of the microhabitat and morphological variables.

	Canonical variables			
	Morphological		Habitat	
	CV_1	CV_2	CV_1	CV_2
Morphological variables				
SVL	0.21	-0.43	-0.04	-0.44
Lamellae	0.30	0.10	-0.03	-0.31
Forelimb	-0.81	-0.57	-0.70	-0.20
Hindlimb	-0.53	0.08	-0.41	-0.11
Tail	-0.15	0.69	-0.04	0.33
Microhabitat				

variables				
Perch height	0.21	-0.49	-0.08	-0.99
Perch diameter	-0.64	-0.44	-0.99	-0.33

The results of the canonical correlation analyses strongly support seven of the predictions of the habitat matrix model (Tables 6.4-6.6). The remaining two predicted relationships were not supported by our data, although the trend was in the predicted direction.

Prediction 1: species using low perches will jump more frequently. The second canonical correlation between behavior and habitat supports this prediction (R = 0.41, p = 0.04; Table 6.3, Figure 6.1b). Behavior canonical variate 2 (CV2) is correlated with jumping frequency and habitat CV2 is negatively correlated with perch height (Table 6.4).

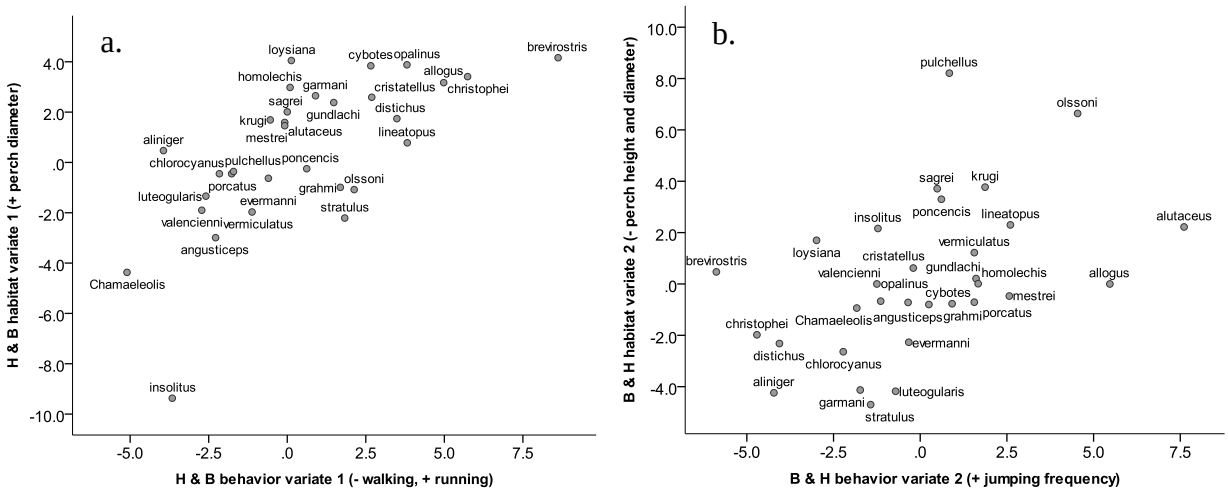

Figure 6.1 (a). In the first canonical correlation between habitat and behavior, species found on narrow perches tend to walk, as outlined in prediction 4, while species found on broad perches tend to run (prediction 7). (**b)** In the second canonical correlation between habitat and behavior, species living on dense, low perches, have a tendency to jump (prediction 1).

Prediction 2: species that jump frequently will have forelimbs that are short and longer tails and hindlimbs. The second canonical correlation between behavior and morphology supports this prediction (R = 0.64, p = 0.01; Table 6.3, Figure 6.2b). In this correlation, morphology CV2 is

correlated with forelimb length and negatively correlated with tail length, whereas behavior CV2 is negatively correlated with jumping frequency and total movement rate (Table 6.5).

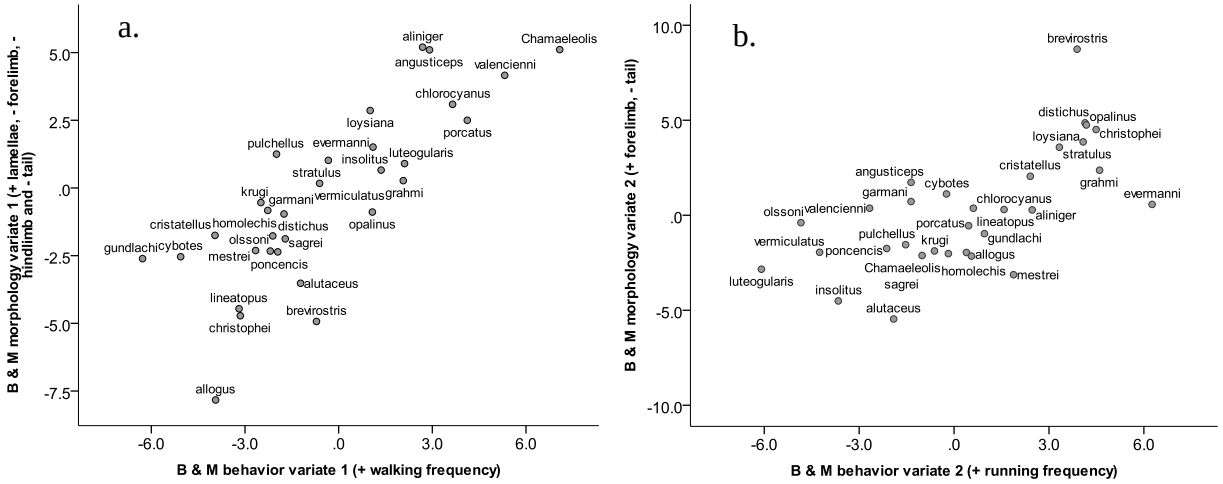

Figure 6.2 (a) In the first canonical correlation between behavior and morphology, species that walk frequently have shorter forelimbs and hindlimbs, consistent with prediction 5 of the HMM. Walking species also have more lamellae. **(b)** In the second canonical correlation between behavior and morphology, species that jump and move infrequently have shorter forelimbs and longer tails , consistent with prediction 2.

Prediction 3: species using low perches will have longer tails and hindlimbs and shorter forelimbs. Although the predicted trend is present in the second canonical correlation between habitat and morphology, this canonical correlation is non-significant thus this prediction is not statistically supported by our analyses (R = 0.47, p = 0.08; Table 6.3, Figure 6.3b).

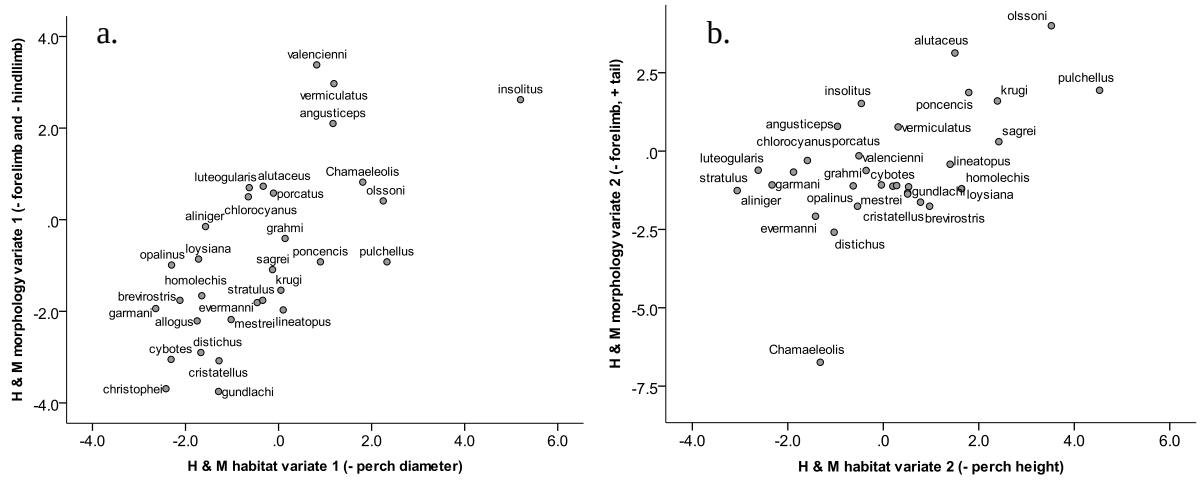

Figure 6.3 (a) In the first canonical correlation between habitat and morphology, species that use narrow perches have shorter limbs (prediction 6). (**b)** In the second canonical correlation between habitat and morphology, species that use dense, low perches tend to have longer hindlimbs and tails (prediction 3), although this relationship is not significant in our dataset. This is true whether the unusual *Anolis chamaeleolis* is included or excluded from the analysis.

Prediction 4: species that use narrow perches will walk more. The first canonical correlation between behavior and habitat supports this prediction (R = 0.67, p = 0.001 ; Table 6.3, Figure 6.1a). Behavior CV1 is negatively correlated with walking rate, while habitat CV1 is correlated with perch diameter (Table 6.4).

Prediction 5: species that walk frequently will have shorter limbs and more lamellae. The first canonical correlation in the comparison of behavior and morphology supports this prediction (R = 0.79, p < 0.001; Table 6.3, Figure 6.2a). Specifically, the first canonical variate for behavior (CV1) is correlated with frequent walking and a high overall movement rate, while CV1 for morphology is correlated with shorter limbs and more lamellae (Table 6.5).

Prediction 6: species that use narrow perches will have shorter limbs. This prediction is supported by the first canonical correlation between habitat and morphology (R = 0.84, p < 0.001; Table 6.3, Figure 6.3a). Habitat CV1 is negatively correlated with perch diameter,

whereas morphology CV1 is negatively correlated with limb length and positively correlated with SVL and lamellae number (Table 6.6).

Prediction 7: species that use broad perches will walk and jump less frequently and run more frequently. The first canonical correlation between behavior and habitat shows this relationship. CV1 is correlated with running frequency, while habitat CV1 is correlated with perch diameter ($R = 0.67$, $p = 0.001$; Table 6.3, Figure 6.1a).

Prediction 8: frequently running species will have long hindlimbs and forelimbs. This prediction is supported by the second canonical correlation between morphology and behavior ($R = 0.64$, $p = 0.01$). The second canonical variate for behavior is correlated with running, while the second canonical variate for morphology is correlated with longer forelimbs.

Prediction 9: species using broad perches will have longer forelimbs and hindlimbs. This prediction is suggested by the first canonical correlation between habitat and morphology ($R = 0.84$, $p < 0.001$; Figure 6.3a). The canonical variate correlated with perch diameter is habitat CV1, and morphology CV1 is positively correlated with both fore- and hindlimb lenth (Table 6.6).

Discussion

Caribbean anoles have become a textbook example of convergent evolution, largely because of the similarities in the locomotor morphology of distantly related species that occupy similar arboreal habitats (Losos, 2009; Futuyma, 1997). The habitat matrix model provides a mechanistic framework for predicting the evolution of these convergent locomotor phenotypes by linking morphology, function, behavior and habitat use (Moermond, 1979a,b; Pounds 1988; Losos, 1990a). Initially proposed by Moermond (1979a,b) and elaborated upon by Pounds

(1988), the model makes nine testable predictions about the interrelationships of these variables. Our examination of these ideas, using by far the largest comparative behavioral data set yet amassed to examine these questions, confirms seven of the nine predictions of the HMM for anoles using field data on the frequency of different locomotor modes of 31 species. Correlations for the remaining two predictions were in the direction expected, though non-significant.

Species that jump

We find good support for a correlation between jumping frequently, long tails and short forelimbs, and the use of low perches (which are typically dense; Pounds, 1988). Specifically, there is a strong relationship between low perch use and increased jumping frequency (Prediction 1; Figure 6.1b) and between jumping and elongated tails plus shortened forelimbs (Prediction 2; Figure 6.2b); an indirect connection between perch height and limb and tail length is suggested by the data but was not found to be statistically significant (Prediction 3; Figure 6.3b).

Although our results indicate that perch height and diameter are related to jumping frequency and morphology, we did not find strong support for all of the predicted morphological variables. Tail and forelimb length were strongly related to locomotor mode and habitat, as expected, but hindlimb length was not strongly correlated with jumping. Tail length and forelimb length are both related to controlling pitch during jumping (Gillis et al., 2009; Legreneur et al., 2012), while hindlimb length is predicted to increase the overall length of the leap (Losos, 1990b). In previous correlational and functional studies, hindlimb length was found to be a strong predictor of jumping ability (Losos, 1990d; Toro et al., 2004). We are unaware, however, of any previous studies demonstrating a relationship between hindlimb length and jumping frequency. Although it seems reasonable to expect that species that jump frequently may also be capable of longer jumps, this is not necessarily true. It may be the case, at least for a portion of the species included

in this study, that the average jump taken in nature is quite short. For example, anoles that live in grasses and shrubs jump frequently but the average gap between perches is small (Pounds, 1988; Losos, 1990c). In this case evolution may not favor especially long hindlimbs, because the ability to jump long distances is not necessary. Indeed, previous work has shown that the maximum jump distances recorded in the lab are almost never observed in nature (Irschick and Losos, 1998). The correlation between jump length and jump frequency, and the differences in morphology that evolve as a consequence of each, would be an interesting avenue for future research.

Species that walk

We find strong support for the connection between frequency of walking, perch diameter, and limb length. Specifically, our data show a relationship between the use of narrow perches and frequent walking (Prediction 4; Figure 6.1a), frequent walking and the evolution of shorter limbs and increased toepad lamellae (Prediction 5; Figure 6.2a), and narrow perches and shorter limbs (Prediction 6; Figure 6.3a). Previous studies have revealed a tradeoff between short legs providing greater "surefootedness" on narrow surfaces versus longer limbs providing enhanced sprinting and jumping capabilities on broader surfaces (Losos & Sinervo, 1989; Losos & Irschick, 1996; Irschick & Losos 1998; Spezzano & Jayne 2004). Likewise, previous correlational studies have found that species found on narrow perches tend to have shorter limbs (Mayer, 1989; Losos, 1990a,b). Taken together, our results and previous work support the prediction that slower but more surefooted walking locomotion is favored over running or jumping on narrow perches.

Species that run

In species that use broad perches, the HMM predicts running will be favored, as will longer limbs. Our data show running species tend to use broad perches (Prediction 7, Figure 6.1a). We also found that species that frequently use broad perches have longer limbs (Prediction 8, Figure 6.3a). Finally, although, the predicted relationship between running and longer limbs (Prediction 9) is suggested by these data, this trend was not statistically significant. The relative weakness of the relationship may reflect the relaxed constraints on locomotion on broad surfaces. Species using narrow perches may be constrained to walking, while species on broader perches can either walk, run, or jump depending on other aspects of habitat such as perch density (Pounds, 1988). The addition of more species to this analysis may help to clarify if the lack of support that we found for the predicted relationship in this case is more likely to be a consequence of the true lack of a relationship or other factors.

Concluding thoughts

Moermond's (1979a,b) framework for understanding the evolution of arboreal locomotion has proven useful to biologists with a variety of taxonomic interests (see Introduction), yet it could potentially be usefully applied to a much broader group. Many species are faced with the challenge of moving through a three-dimensional matrix of vegetation, including some that would not be traditionally defined as arboreal (Moffet, 2000). The idea of the "canopy" in biology has been applied somewhat exclusively to the upper reaches of trees, yet the structure of a forest canopy poses challenges to locomotion that are similar to those imposed by many other habitats (Moffet, 2001). For example, for an insect or small mammal, a prairie is composed of a matrix of grasses that may or may not bear weight. For animals that live in the leaf litter, movement through the habitat may be dominated by dead branches. In aquatic habitats, the architecture of macroalgae are strongly reminiscent of forests. The habitat matrix model may be

a useful way to study the locomotion of insects in grasslands, small mammals in desert scrub, amphibians in the leaf litter, crustaceans in kelp, as well as animal locomotion in a range of other situations.

Chapter 7

Conclusion

In this dissertation, I set out to explore the evolutionary forces that shape the *Anolis* dewlap at multiple levels, from variation among individuals in one population (chapters 1 and 4), to variation among populations (chapter 2) and species (chapter 3). These projects revealed several unexpected insights on dewlap evolution, and suggest multiple avenues for future research to advance our understanding of the *Anolis* dewlap and the evolution of animal ornaments more generally.

In chapter 1, I demonstrate for the first time that dewlap color is currently under selection in *A. carolinensis*, a model species for behavioral research. Surprisingly, my data do not support the classical view of sexual selection in *Anolis*, where male competition for territories determines their access to mates. Several lines of evidence from my study contradict this view: I did not find a relationship between dewlap morphology and male territory; male territories and reproductive success were not related; many females mated with multiple males; and the spatial patterns of mating suggest that females often mate with males other than the male that controls their territory. Another surprise in my data was that a large number of males observed in the study area were observed on only one or two occasions over a four-month period. These "transient" males may represent an alternative male behavioral strategy, and deserve more detailed study.

In chapter 2, I show unexpected variation in the dewlap size, color, and pattern among recently introduced populations of *A. sagrei*. A large portion of this variation could be explained by ecological variation among sites, while relatively little of the variation could be explained by the genetic relationships among populations, suggesting that rapid adaptive evolution of this

ornament may be occurring over very short time scales. The ecological variables that explain dewlap variation suggest that multiple, sometimes conflicting selective forces are shaping dewlap variation, including selection to facilitate species recognition in different assemblages of anoles, sexual selection, predation, and sensory drive. These results suggest that more detailed studies, measuring selection directly among multiple populations, would be fruitful. More generally, these results suggest that the novel environments experienced by introduced populations could lead to changes in ornaments involved in species recognition, perhaps someday leading to anthropogenic speciation.

In chapter 3, I shift from a focus on variation within species to variation among species, and from a focus on male ornaments to a focus on female ornaments. Like male ornaments, female ornaments can evolve in response to social selection (for example, if males engage in mate choice, or to mediate female territorial disputes), species recognition, or sensory drive. Since male and female ornaments share an underlying genetic basis, female ornaments may also evolve as a consequence of selection on males, an idea referred to as the genetic correlation hypothesis. I gathered data on relative dewlap size for both males and females from 228 species to test these hypotheses using a phylogenetic comparative approach. Female dewlap size was correlated with sexual dimorphism and microhabitat preference, but not with community complexity or relative male dewlap size, suggesting that social selection and sensory drive may be responsible for the evolution of the female *Anolis* dewlap. Although these patterns of correlation are suggestive, almost no detailed behavioral studies have been conducted on female communication in *Anolis* to confirm when and how the female dewlap is deployed. This would be an obvious next step to advance our understanding of the evolution of this female ornament.

Chapters 1-3 all explicitly concern the evolution of the dewlap at multiple levels. I find evidence that a number of different types of selection are likely acting on the dewlap and that all of these processes have likely contributed to the spectacular diversity of dewlap colors and patterns in this genus. In part, it may be the complex interaction of these conflicting sources of selection that have resulted in such a remarkable radiation of ornaments. In the future, I would like to use the detailed approach employed in chapter one to characterize selection in multiple populations and species to eludidate how variation in selection over space and time contribute to dewlap diversification. It would be especially instructive to combine detailed data on mating patterns, behavioral interactions among different species of anoles, and observations of predation among populations to isolate the action of each type of selection within and among populations. The enormous effort required to collect this sort of data may not be feasible at present, but future advances in the technology to track individual anoles over space and time, to record their proximity to other individuals of their own species and others, to measure their reproductive success, and to identify when and by whom they are eaten may make this possible in the future.

Another future aim would be to establish that dewlap color variation is the cause of differences in reproductive success among individuals by manipulating dewlap color to observe how this alters reproductive success and the outcome of behavioral interactions among individuals, both of the same species, other anoles, and predators.

More generally, anoles are an excellent example of a widely observed phenomenon that some of the most species rich groups of animals are also groups that show lavish variation in ornaments used in visual communication. A variety of authors have suggested that the connection between ornament diversity and species diversity imply that sexual selection is a driver of speciation (see the introduction of chapter 2 for a review of these studies). The results

presented in chapters 1-3 suggest an alternative: that it is the interaction between many forces such as sexual selection, species recognition, predation, and habitat variation, acting concurrently in concert and in conflict, that may lead to the greatest diversity of both species and ornaments.

In chapters 4 and 5, I explore questions about the behavior and evolution of anoles that are less directly related to the dewlap. The focus of chapter 4 is on a remarkable case study, *Anolis limifrons*. Unlike most anoles, males and females of this species form stable persistent pairs in nature that last the lifespan of the animals. I found evidence that males and females mate assortatively by size in this species, but that variation in dewlap size or body dimensions did not explain which animals would fail to form pairs. The next step to understand this unusual behavior would be to use genetic analysis of parentage to determine if the pairs observed in nature are also mating monogamously, and whether animals that don't pair are still able to successfully reproduce.

In chapter 5, I find evidence in support of the habitat matrix model, a set of predictions on how microhabitat variation, morphology, and locomotor mode coevolve in arboreal species. While I find strong correlations among morphology, behavior, and habitat use among species suggesting coevolution between these groups of traits, the data and methods currently available do not reveal the order of trait divergence in this coevolutionary complex. In the future, it would be interesting to try to determine whether changes in microhabitat use lead to changes in morphology and behavior, or whether behavior restricts microhabitat use and morphology, or if morphology determines both locomotor behavior and microhabitat use. One approach to finding these connections might be to look at divergence in these traits among recently introduced populations such as those considered in chapter 2, to see if these traits are changing predictably

and in some deterministic order among populations that are likely currently undergoing adaptive evolution.

Together, these chapters offer new insight on the ecology and evolution of *Anolis*, particularly on the evolution of the dewlap. The data presented here suggest that selection is currently acting on the dewlap, probably in multiple conflicting directions. These results suggest that further studies on *Anolis* dewlap evolution are likely to prove fruitful, both for understanding the specific evolution of the dewlap and perhaps for understanding more generally the processes that drive ornament diversity among species.

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Appendix A

Supplementary materials to Chapter 3.

Table S1. Correlations between raw male and female dewlap color as measured in the center of the dewlap, and sexual dichromatism (SDC). PCA color variables could not be used because edge measurements were not available for females.

		R^2	p
Males	**UV (UVWS)**	-0.87	<0.001
	Blue (SWS)	-0.91	<0.001
	Green (MWS)	-0.94	<0.001
	Red (LWS)	0.92	<0.001
Females	**UV (UVWS)**	-0.18	0.50
	Blue (SWS)	-0.25	0.35
	Green (MWS)	-0.47	0.07
	Red (LWS)	0.31	0.24

Additional habitat variables

As part of separate research projects, we collected data on different characteristics of the habitat at several sites included in this study. The habitat variables we considered include the height and diameter of the perch where a lizard was first observed, measured to the nearest millimeter, and the percent canopy cover at the perch site. Canopy cover was measured with reference to a spherical densiometer held about a meter from the body at the location where the lizard was observed. For all sites where these variables were recorded, a minimum of 50 perches used by

adult males were measured. To determine if our climate estimates were related to the structure of the vegetation on the ground, we first arcsin-square root transformed percent canopy cover, then calculated the Pearson correlation coefficients for the habitat variables extracted from the Worldclim dataset versus the habitat variables measured in situ.

Table S2. Correlations between habitat variables measured on the ground (percent canopy cover, perch height, and perch diameter) and habitat estimated from WorldClim data.

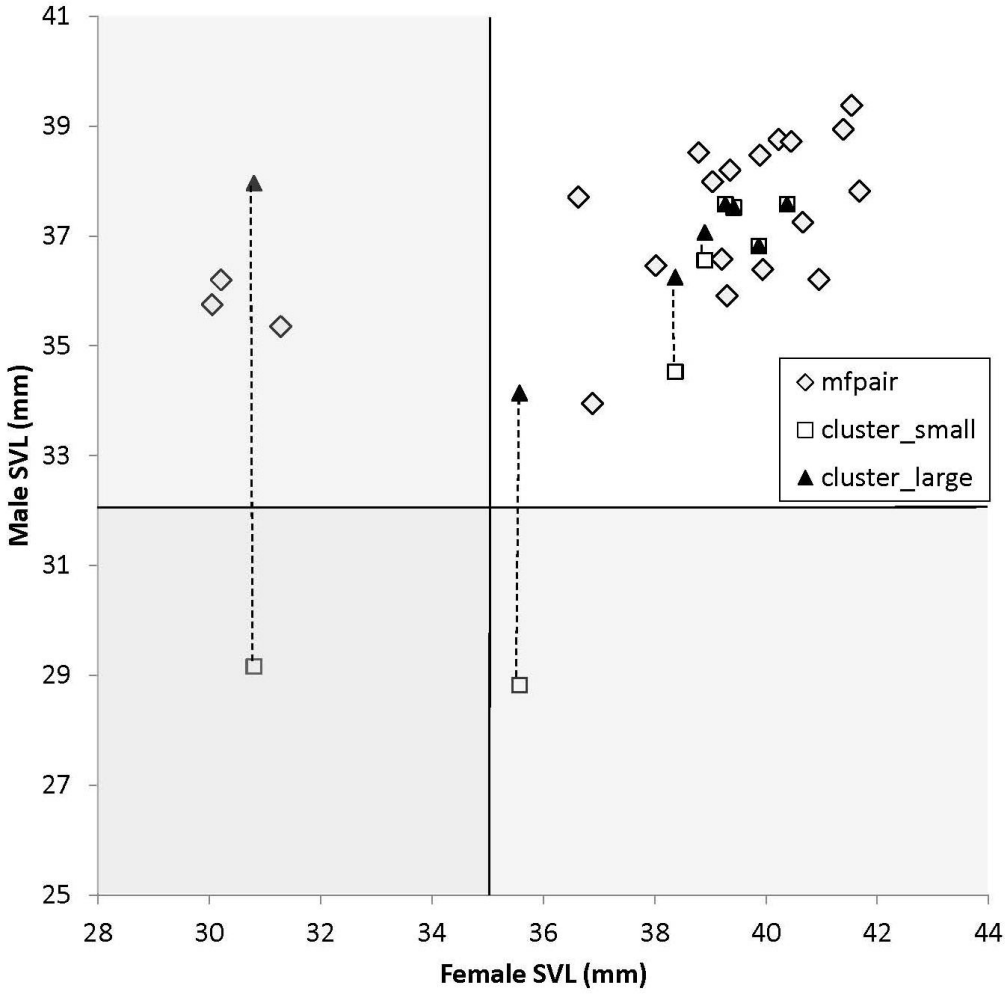
	Populations compared	**Climate PC1 R^2**	**Climate PC1 p**	**Climate PC2 R^2**	**Climate PC2 p**
Percent canopy cover	14	0.09	0.76	-0.20	0.50
Perch height	10	-0.36	0.31	0.34	0.33
Perch diameter	12	-0.24	0.45	0.28	0.38

Appendix B

Supplementary materials to Chapter 5

Table S3. Morphological comparisons between paired and unpaired animals

Trait	Males: paired vs. solitary	Females: paired vs. solitary
SVL	F = 1.13, d.f. = 1, p = 0.29	F = 0.59, d.f. =1, p = 0.45
Tail length	F = 0.19, d.f. = 1, p = 0.66	F = 0.12, d.f. = 1, p = 0.73
Forelimb length	F = 2.95, d.f. = 1, p = 0.93	F = 0.45, d.f. = 1, p = 0.51
Hindlimb length	F = 0.01, d.f. = 1, p = 0.91	F = 0.41, d.f. = 1, p = 0.53
Head length	F = 1.18, d.f. = 1, p = 0.28	F = 0.03, d.f. = 1, p = 0.87
Head width	F = 0.02, d.f. = 1, p = 0.89	F < 0.01, d.f. = 1, p = 0.96
Head depth	F = 2.84, d.f. = 1, p = 0.10	F = 0.87, d.f. = 1, p = 0.36
Dewlap area	F = 1.62, d.f. = 1, p = 0.21	N/A



Supplementary Figure S.1. Correlation between body size of paired males and females, showing all data. Solid vertical and horizontal lines indicate size at sexual maturity for females and males, respectively. Pairs falling in the grey areas were excluded from the main analysis because one or both members of the pair were not sexually mature; in this case the correlation between the body size of pairs was R = 0.50 (p= 0.039). If all pairs (grey diamonds) are included regardless of size, the correlation between males and female body size is R = 0.57 (p= 0.009). Empty boxes and black triangles represent potential pairs of animals that were excluded because they were found in clusters. The empty square represents the size of the smallest male in a cluster and the black triangle represents the largest male in a cluster. Males found with the same female are connected with a dashed line. If the correlation between males and females is calculated including animals that belong to clusters, the correlations is R = 0.64 (p= 0.001) if the larger male is used and R = 0.53 (p= 0.010) if the smaller male is used. If all animals are included in the analysis, including both the larger and smaller male found in a cluster and animals that are not sexually mature (all data shown) the correlation is R = 0.52 (p= 0.001). In summary, regardless of how the data are treated, a significant positive correlation exists between the body size of paired animals.